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Estimation of Weaning Patterns in the Elite Meroitic Population (8-B-5.A) from Sai Island, Sudan Using Stable Nitrogen and Carbon Isotopes

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ESTIMATION OF WEANING PATTERNS IN THE ELITE MEROITIC POPULATION (8-B-5.A) FROM SAI ISLAND, SUDAN USING STABLE NITROGEN AND CARBON ISOTOPES

by

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ABSTRACT

This research explores dietary patterns of elite non-adults from the Meroitic period (300 BC – AD 350) located in Sai Island, Sudan. The cemetery (8-B-5.A) is believed to have been in use during the 1st and 2nd centuries AD. Non-adults were chosen because they offer a unique, and often ignored, perspective into customs of past populations. Children require significant energy, which impacts how society feeds and cares for their young. Knowledge of their elite status in society will be consider to explore how this subset of the population may have differed in behavior. A significant factor of child life is access to food. One way to examine this aspect of past populations is to reconstruct infant weaning and feeding patterns using stable isotope analysis. This study analyzes stable nitrogen and carbon isotopes from the bone collagen of 31 non-adult individuals, ranging in age from 38 weeks to 16 years. Stable nitrogen isotopes are used to identify if the infants have higher trophic levels than their mothers, an indication of breastfeeding, and stable carbon isotopes are used to identify potential weaning foods. Taken together, and compared against sample ages, the longevity of the weaning process is considered, particularly when compared to adult male and female isotope values. Isotopic results indicate that non-adults in this population were weaned between 2-4 years of age and weaning foods were a combination of C_3 and C_4 plant food sources. Significant variation in isotope values was found in the younger non-adults, which indicates a potential difference in weaning style. These results can be built upon in further studies to further explore the motives of elite Meroitic parents.

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CHAPTER ONE: INTRODUCTION

Humans have always employed unique adaptations to not only survive, but thrive, which has manifested in the houses we build, the clothes we wear and how we consume nutrients. Understanding how and why these aspects of life evolve can shed light on key aspects of humanity. One of these facets is diet, particularly the diet of children, which is unique due to their reliance on the adults in their community. One specific process of their diet, weaning, is shaped by many societal and environmental circumstances. This is because breastfeeding can be key to a child's survival, especially where there is limited healthcare (Moffat and Prowse 2018). Patterns in the weaning process can be limited and altered due to availability of resources, potentially due to economic status and cultural norms outlined by influential members of society (Moffat and Prowse 2018).

This research focuses on dietary patterns of a population from an elite Meroitic (1st and 2nd century AD (Francigny 2014)) cemetery (8-B-5.A) located on Sai Island in northern Sudan. The sample consists of non-adults aged from 38 gestational weeks to 16 years old. In addition, a sample of the adults in the population was used as a baseline for a comparison to the non-adult values. Sai Island, noted for its Ottoman fort and continued occupation over millennia, holds two important geological and geographical advantages. Firstly, the island is located at a significant elevation above the Nile River, making it immune to the annual inundations of the Nile flood waters. This means that the island can be occupied year-round, and from an archaeological perspective both material culture and skeletal remains are preserved. Secondly, its location is strategic in that it allowed for control of the Nile River on the frontier of the Egyptian and Nubian Empires (Francigny 2014).

Although food has long been a focus of archaeological study, until recently there has been a notable lack of research surrounding children, and particularly how their diet may have factored into daily life (Halcrow and Tayles 2011). Due to an increased use of feminist theories in studies in the 1970s, the lives of past children begun to enter the research agendas of archaeologist and bioarcheologists. By the 1990s studies of children in the past finally gained credibility (Halcrow and Tayles 2011). The analysis of younger non-adults is essential because it can shed light on the customs and lifestyle of a society (Halcrow and Tayles 2011). Further, a comparison of young non-adult isotope values to contemporaneous adults and older non-adults can aid in the interpretation of culturally driven divisions, and give an understanding of how dietary patterns evolved across age groups, and the consistency of these changes.

One particular aspect in the study of non-adults is the weaning process. Weaning is when children slowly stop exclusively breastfeeding and begin to supplement their diet with other foods. The weaning process is complete when children subsist completely on food sources other than breast milk. The age when weaning begins and the length of the weaning process is connected to a variety of factors, including the economic and health resources available to mothers, and the number of children desired (Kamp 2001). Of particular interest in this study is that the population from which the samples are drawn has been identified as elite. This may influence access, not only to more nutritious foods, but to paid assistance, such as wet nurses. Previous studies have noted a difference in feeding patterns between class (e.g. Katzenberg and Pfeiffer 1995). The potential of this influence will be acknowledged in the analysis.

In addition to the age of weaning, the types of foods consumed during and after weaning can reveal further morbidity and mortality information. This is based on the idea that specific foods would be more successful than others in sustaining the non-adult individuals. Eerkens et al. (2018) suggests the importance of identifying weaning foods for future research, because when it is combined with life history analysis, it may be possible identify which foods had better outcomes for survival. This type of analysis could be conducted by comparing the isotopic data patterns between the individuals who survived into adulthood and those who did not. Eventually, according to Eerkens et al. (2018), this data could be compared between groups within the population (e.g. elite and non-elite) and other contemporaneous sites.

There have been many studies regarding weaning patterns in past populations (Fogel et al. 1989; Fuller et al. 2006; Katzenberg and Pfeiffer 1995). Initially, weaning was studied through the presence of certain pathologies associated with weaning stress such as enamel hypoplasia and cribra orbitalia (Katzenberg and Pfeiffer 1995). Currently, however, stable isotope analysis has become the main methodology for providing direct evidence of weaning practices (Fuller et al. 2006; Dupras et al. 2001). Stable isotope analysis of bodily tissues provides direct evidence of dietary input. In the case of weaning studies, stable carbon and nitrogen isotope analysis of tissues such as bone collagen, dental dentin, and soft tissues such as hair and nails are commonly used to assess the weaning process (Katzenberg and Pfeiffer 1995; Dupras et al. 2001; Fogel et al. 1989;). The focus of this research will be on the analysis of stable carbon and nitrogen isotopes from bone collagen.

This research is focused on three main questions. The first question is, “At what age were the children in the sample weaned?” Based on previous studies of this population

(Eerkens et al. 2018), it is hypothesized that the weaning process will end around 2.5 years of age. The second question is, "What foods are being used to wean the children?" Using the same resource (Eerkens et al. 2018), it is hypothesized that the amount of C_4 and C_3 food sources in their diet will be varied, but with more C_3. This is based on the evidence that the foods that are consumed by humans are more commonly C_3 (DeNiro 1987). The last question is, "What factors are believed to influence this timing?" Some of the influences mentioned in previous work (Halcrow and Tayles 2011; Tsutaya et al. 2015) relate to socio-economics, gender, health issues, and the choices of individuals mothers. The answers to these questions will help to understand the population, and potentially Meroitic culture, and their belief regarding infant feeding practices. Additionally, it will speak to larger questions about childhood and dietary patterns.

While the focus of this research is biological, elements from other sub-fields of anthropology will be utilized including archaeology and sociocultural anthropology. Without archaeology, the data loses all context and without an understanding of social theories, the analysis would be limited. This project will incorporate research surrounding childhood, biocultural, and life history theory. In addition, this research will include a review of the literature pertaining to the connection between weaning patterns and different economic, social, and demographic aspects of society.

This thesis is divided into six chapters. Chapter two is the literature review, which explores background information regarding Meroitic culture, childhood, weaning, and stable isotopes. Chapter three contains the materials and methods, and will explore the specific samples, age estimations, and the steps for collagen extraction and analysis. Chapter four is the

results section, including results for sample preservation, and the results of the carbon and nitrogen isotope analyses as compared by different age categories. Chapter five is the discussion of the previously presented results, which includes weaning patterns, estimates of timing and estimate of complementary food used during weaning, and the variables that could have influenced them. The last chapter is the conclusion, and summarizes the key findings of this research, considers the limitations of the sample, and explores avenues for future research.

CHAPTER TWO: LITERATURE REVIEW

The goal of this chapter is to highlight pertinent previous research on childhood, particularly those concerning weaning and stable isotope analysis, within the context of Meroitic life. This chapter begins with a description of the Meroitic period (300 BC – AD 350) and the research site, Sai Island. After there will be a consideration of how archaeological research related to children and childhood has developed over time in order to demonstrate the importance of children in their communities and what can be learned. Following this is a detailed description of the important biological aspects of weaning and how they factor into the analysis. A section on age categories follows this, as how non-adults are placed into age categories has a big impact on how the data is interpreted through a theoretical lens. Lastly, this leads into the literature review on stable isotopes and stable isotope analysis, particularly bone collagen, and nitrogen and carbon isotopes.

Ancient Nubian and Sai Island Background Information

Ancient Nubia and Meroe

The Kingdom of Nubia was located in the southern parts of modern-day Egypt and northern Sudan. Nubian settlements remained close to the Nile River because it provided inhabitants with water to support agriculture (Lacovara 2012). Nubia is divided into two main sections along the Nile River: Lower Nubia in the north and Upper Nubia in the south (Lacovara 2012). The Nile River in Nubia is also divided by six different cataracts (Lacovara 2012), making it difficult to navigate by boat. Sai Island is located in Upper Nubia, between

the second and third cataracts. The history of ancient Nubia is divided into approximately 11 time periods (Table 1). As mentioned previously, the samples to be analyzed for this research are from the Meroitic period (300 BC – AD 350), specifically the 1st and 2nd centuries AD.

Table 1 Ancient Nubian Occupations in Upper and Lower Nubia (Derived from Hawass 2012)

Date	Upper Nubia	Lower Nubia	Egyptian Period Influences
Before 3050 – 2685 BC	Classic A-Group Terminal A-Group	Pre-Kerma	Predynastic Period – Archaic Period
ca. 2685 – 2008 BC	C-Group Ia, Ib	Early Kerma	Old Kingdom – First Intermediate Period
ca. 2008 – 1685 BC	C-Group IIa, IIb	Middle Kerma	Middle Kingdom
ca. 1685 - 1550	C-Group III	Classic Kerma	Second Intermediate Period
ca. 1550 – 1077 BC	Egyptian Occupation	Egyptian Occupation	New Kingdom
ca. 1076 – 723 BC	Independent Nubian cultures	Independent Nubian cultures	Third Intermediate Period
ca. 722 - 332 BC	Napatan	Napatan	Later Period
350 BC – AD 350	Meroitic	Meroitic	Ptolemaic - Roman
AD 350 – 641	Post Meroitic	Post Meroitic	Roman
AD 641 – 1400	Christian	Christian	Islamic
AD 1400	Islamic	Islamic	Islamic

During the Meroitic period, the capital of Nubia was in Meroe, and the Kingdom consisted of Lower Nubia and potentially lands further down south (Fisher 2012). Due to the strategic location of Lower Nubia, there were many power struggles over this area throughout history, and this was also true during the Meroitic period (Fisher 2012). In AD 350 the Meroitic kingdom ended as it had been taken over by the Axumite Empire (Fisher 2012). At this time Lower Nubia entered in to the Post-Meroitic period, followed by the Christian period (AD 641-1400).

The Meroitic period is marked by the change of burial locations of kings. Originally buried in Napata, the elite royalty mortuary cult moved to Meroe (Fisher 2012). The specific cultural catalyst for these changes is not definitively known (Edwards 2004). During this time there was advancement in irrigation, agriculture, and storage (Fisher 2012). Continual settlements on the riverbanks around this region have been found to date back to at least the 8th millennium BC (Haaland 1995). Throughout the Meroitic period, however, communities used varying techniques for gathering food dependent on their specific environment (Edwards 2004). Overall there is limited information about the agricultural and herding patterns employed (Edwards 2004).

Some food of importance has been identified as sesame, date palms (Fisher 2012), and grains such as wheat, barley, millet, and sorghum (Edwards 2004). Sorghum has been identified as particularly important in the Meroitic period (Edwards 2004). The use of barley was thought to decrease in comparison to its use in the Napatan period (Edwards 2004). The types of food people ate may have differed due to social class and position (Haaland 2014). The cultivation of agricultural fields most likely relied on the annual flooding of the Nile River. When the water receded, the soil would be fertilized for their crops (Edwards 2004). This will be important to acknowledge when interpreting the nitrogen ratios. Additional irrigation methods, such as the "*saqui* waterwheel," may have been used, but more evidence is needed (Edwards 2004: 165). This technology was particularly important for allowing a wider variety of crops to be cultivated (Edwards 2004), allowing for improvement on wheat and sorghum crops, and the introduction of others like pearl millet (Edwards 2004).

While the potential livestock consumed includes “sheep, goats and cattle,” (Edwards 2004: 165), cattle were particularly important as both food and a symbol of elite status (Edwards 2004; Haaland 2012). Cattle, in addition to giraffes, ostriches, humans, and sorghum plants, have been found as images on pottery (Edwards 2004: 172). Since the population being studied is considered elite, it is probable they were consuming protein from cattle. There was also potential interaction between nomadic pastoral groups and the more permanent settlements (Edwards 2004), and their contribution to food resources should be considered when interpreting stable isotope data.

Additionally, there was a “Roman–Hellenistic” influence on the food sources due to contact through trade and proximity (Haaland 2014). While there is historical evidence demonstrating contact between Meroe and larger powers, such as Egypt, the greatest evidence of trade comes from burial artifacts such as furniture, wine and oil bottles, and ceramics (Edwards 2004). Most of these objects were found in elite burials, and were even described by Edwards (2004) as “a royal monopoly,” (168). Additionally, there is archaeological evidence that pepper was important during the Roman period (Cappers 2006), and may have been made available through trade (Haaland 2012). Pepper would have mostly likely been used for important events or be connected with social standing (Haaland 2012).

One notable aspect of Nubian Sudanese culture is the consumption of beer. This not only served an important social function, but also a medical one (Armelagos 2010). Bassett et al. (1980) first noted tetracycline-labeled human bone from Sudanese Nubia (circa AD 350) that were identical to patterns noted in modern humans who were treated with the antibiotic tetracycline. The authors note that the beer making process in ancient Nubia, particularly

storing grain in ceramic jars, encouraged the growth of the bacteria *Streptomyces* which produced tetracycline. The consumption of beer is thus hypothesized as being the delivery method for tetracycline which then labeled the bone. The concurrent effect on health is unknown. While the samples used in Bassett et al. (1980) are later in date compared to those used for this research, the consumption of beer has been recorded in the Meroitic period (Haaland 2012), and beer jars have been recovered from the 8-B-5.A cemetery (David, Pers. Comm). It is worth considering what types of grains were used to make beer, and whether beer would have been consumed by children during the weaning process or by the mothers during illness brought on by pregnancy.

Sai Island and Cemetery 8-B-5.A

The research samples originate from Sai Island in northern Sudan located on the Nile River. Sai Island is unique because it contains the remains of many distinct temporal cultural populations. The individuals studied in this research come from the archaeological site 8-B-5.A, located on the northeast side of the island (Figure 1). This site is archaeologically complex, presenting remains from three temporally distinct populations, the elite Meroitic cemetery (300 BC – AD 350), a Christian cemetery (AD 600 -AD1400), and an Islamic population (AD 1317 – present), each successively using the same geographical location for their burials.

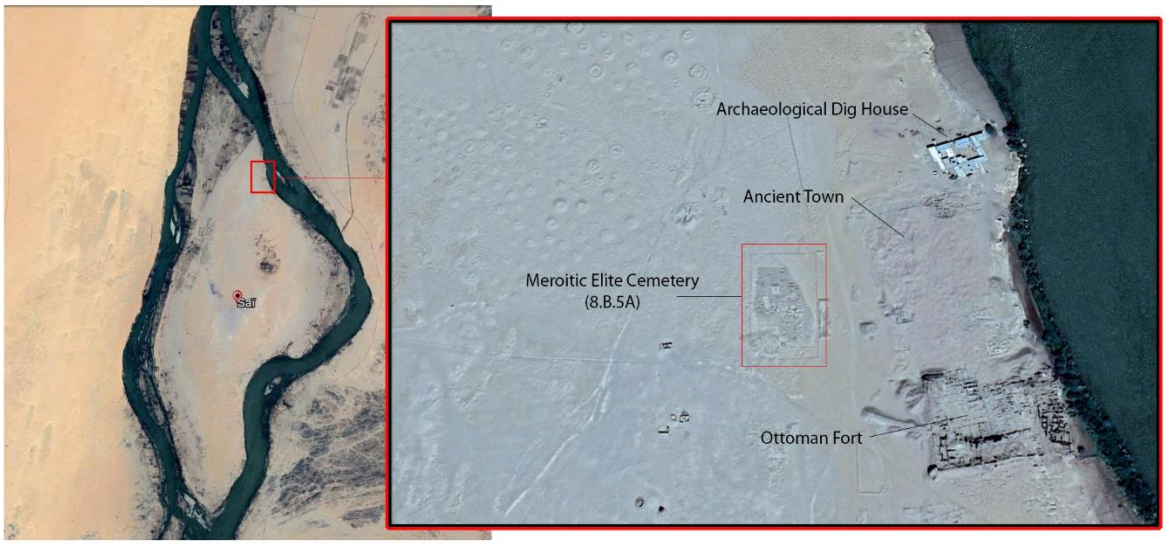

Figure 1 Satellite and drone imagery of Sai Island, on the left, red square highlighting the location of the elite Meroitic cemetery (8-B-5.A). Drone imagery courtesy of the Sai Island Archaeological Mission.

It is important to acknowledge that the population being studied is considered, from an archaeological context, to have been elite. The elite occupants of the city of Meroe, the Meroitic capital, built funerary complexes with stone or masonry pyramids reflecting the Egyptian style, (although this changed to red brick or "rubble covered with plaster" after AD 50 [O'Connor 2012, 116]) as the city was home to the royal mortuary cult (Fisher 2012). While these pyramids primarily contained royalty, it is believed that commoners were also buried in these complexes (O'Connor 2012).

This same burial style is reflected in the Sai Island elite Meroitic necropolis, 8-B-5.A (Francigny 2012). In this case, all pyramids are constructed from mudbrick and filled with rubble (Francigny 2012). The specific cemetery of interest, 8-B-5.A, was first thought to be elite due its location on a hill where it could always be seen (Francigny 2014). While most of the graves were heavily looted, some remaining artifacts support the occupants being elite

(Francigny 2014). This include pottery and fabrics (Francigny 2014), and materials made from glass and ivory (Francigny 2008), which are objects that suggest a higher economic status. Original bioarcheological research on the Meroitic elite from Sai Island suggests that the Meroitic elite population was relatively healthy with few markers of stress or pathology, and that they had protein and iron rich diets (Francigny 2010).

Archeological evidence indicates that the elite Meroitic cemetery 8-B-5.A, on Sai Island, was first used during the 1st Century AD (Francigny 2009). Francigny (2009) discusses how the Meroitic phase of the necropolis consists of pyramid structures with subterranean tombs (including a descenderie and burial chamber) located under the pyramids and throughout the cemetery. During the Christian period, adult Christians and older juveniles were buried in a cemetery toward the west and surrounding the Meroitic cemetery. The Christians used the Meroitic elite graves, particularly the entrance or descendaries, to bury infants and young children. It is believed that this was because the Meroitic graves were easy to dig up due to their "sandy" composition (96). Thus, burial practices were used to distinguish Christian burials from Meroitic burials. The site is small, and it is believed that the creation of this cemetery was associated with a new religious elite, and that only a privileged few or the elite were buried there.

Archaeological sites on Sai Island represent the different populations of Nubian history (Francigny 2009). At 12 kilometers long and 5.5 kilometers wide (Yellin 2012) Sai Island is the largest island in the Nile Valley and has been depicted as a "strategic area" (Francigny 2014: 797). The climate of the area is dry (Yellin 2012), presenting an excellent environment for archaeological preservation. The first explorative contact was in 1842 by the Prussian

Royal Expedition lead by Richard Lepsuis (Yellin 2012). It was not until the 1940s, however, that Anthony Arkell officially conducted archaeological survey on the island (Yellin 2012). Since then, Sai Island has been under the concession of the French Antiquities Service with intermittent excavations conducted by Jean Vercoutter (1954-1957), Francis Geus (1993-2004), and Didier Devauchelle and Florence Doyen (2006-2011) (Yellin 2012). Currently the site is under the direction of Vincent Francigny (2011 -present) (Francigny 2009).

Biocultural and Life History Theories

As mentioned previously, there are two theoretical frameworks that will be employed in the interpretation of this research. The first theoretical approach is called biocultural theory, which recognizes that both social and biological factors can influence a person's life (Inglis and Halcrow 2018). The second theoretical approach is life history theory, which utilizes evolutionary perspectives to understand different cultural adaptations related to life cycle stages such as infant feeding and weaning patterns.

Bioarcheological research focused on diet commonly uses both of these theoretical approaches to interpret diet through the life cycle. This is because food has both social and economic factors, while also being necessary for growth, development, and survival (Moffat and Prowse 2018). Additionally, having access to proper nutrition can impact an individual in the long term (Moffat and Prowse 2018) and may change their skeletal structure. Thus, the environment, which also shapes diet, social and economic factors, can affect an individual's health, and directly influence their skeletal system (Inglis and Halcrow 2018). Through this

framework, the data resulting from this research will aid in understanding the outside factors that may influence the diet of the children from this elite population.

The main way to understand how outside factors affect skeletal remains is to contextualize them within the understanding of human biological plasticity (Sofaer 2013). This idea was previously connected to growth when studying children, especially infants (Bogin 1999), and dates to Franz Boas who demonstrated that a person's growth was not static but influenced by their environment (Lesser 1968). While the concept of plasticity is still being studied, the particular research aims have changed to consider questions such as how and at what point in human life is plasticity shaped (Agarwal and Beauchesne 2011). This question relates directly to this research, specifically because it has been shown that nutrition in early life can impact an individual's lifetime immunity (Inglis and Halcrow 2018). Understanding biological plasticity in relation to the high energy demands of infancy and disease in early life can help investigators understand the impacts of environment and geographic location on the human skeleton (Inglis and Halcrow 2018).

Another consideration to make when interpreting the lives of non-adults and adults is the Barker Hypothesis which postulates that there is a direct causal relationship between intrauterine growth retardation, low birth weight, and/or premature birth and health issues during adulthood. As such, nutrition has been shown to play an important role during early life (Weaver 2011). In addition, new ideas pertaining to epigenetic factors, particularly those involving mother and child, have been explored by Gowland (2015). These factors, combined with the Barker Hypothesis, helped to inspire the Developmental Origins of Health and

Disease (DOHaD) hypothesis, which postulates that everything in early childhood can affect how you are as an adult (Gowland, 2015).

Bioarcheological study of the skeleton can provide us with an understanding of how our physiology interacts with the environment. For example, "The transverse and anterioposterior diameters of the neural canal are 'locked-in' by five years of age…while vertebral body height may continue growing into early adulthood, " (Gowland 2015, 532). Essentially, by certain ages different types of traits become static, and understanding when those time periods are could be important for understanding overall health of the population. We are able to examine questions such as, is there a critical age when individuals must receive necessary nutrient to thrive later in life?

Two other theoretical frameworks that can be used to study children are the life course approach and life history theory, which are sometimes conflated (Inglis and Halcrow 2018). Inglis and Halcrow (2018) differentiated the two by stating life course approach is looking at the life of an individual not just one stage of their life and life history is an understanding that how a human evolved is connected to different developmental stages. The life history approach will be used in this research as the main focus is on nutrition at the specific point in the child's life and not the whole life, which is due to the cross-sectional nature of the sample.

Developed out of evolutionary theory, life history theory can provide a framework for understanding social responses to biological aspects of life (Inglis and Halcrow 2018). The foundational idea of this theory is that the body has a finite amount of energy that must be used for growth, reproduction, and maintenance, with the main goal being reproduction (McDade 2003). Specific ecological and physiological limitations will determine an organism's survival

strategy. McDade (2003) uses this framework to discuss growth by claiming children who have more access to food may have more energy to fight off diseases and to invest in growth. Life history is an important framework for this research because it aids in the understanding of the importance of a specific stage of life in this community, and if any ecological or cultural aspects influenced the diets of children, specifically, how long they were breastfed.

A History of Theory in Childhood Research

Human non-adults, individuals under the age of 17, offer unique insight into the structure and beliefs of past communities (Halcrow and Tayles 2011). Prior to the 1970s, females and children did not receive much attention in archaeological studies (Baxter 2008). Children were especially ignored because many researchers believed they did not have a significant impact (Lewis 2007). Additionally, archaeological practices and preservation issues can play a big role in how non-adults are represented in the archaeological record (Lewis 2007). These views changed in the 1970s when feminist theory influenced literature and research aims (Baxter 2008) and the economic contribution of women and the impact of children began to be explored (Halcrow and Tayles 2011).

One of the first books on the social aspects of children, "Centuries of Childhood", was written by French social historian Phillipe Aries (1962). The publication faced strong critique due to its universal view of childhood (Lillehammer 1989), and concerns about methodology and evidence (Wilson 1980). Aries' 1962 publication, however, is considered important for creating a foundation for understanding childhood as a constructed social stage (Halcrow and

Tayles 2011). This essentially means that the norms and stages of childhood are manufactured by the individual's culture.

It is important to note that modern Western ideas of children are not universal through time and culture (Lewis 2007). Aries (1962) initially argued that childhood is a modern phenomenon, and that children in the past were treated as adults due to higher rates of child mortality. Later studies (e.g., Swanson 1990; Shahar 1992) moved away from a social evolution view, presenting evidence of children being considered as children in the past (Lewis 2007). This view is relevant because it demonstrates the need to not assume how children were viewed and treated in the past.

While Aries' (1962) article was influential, it was Lillehammer's (1989) article, "A Child is Born: The Child's World in an Archaeological Perspective," that is credited as the seminal publication for studying children in the archaeological record. Lillehammer (1989) advocated for understanding the difference between a "child's world" and the "adult's world", iterating that each has a different perspective, which will yield different questions. She argues that the "child's world" has always been present in archaeological study, however, it is only since the 1970's that researchers have deliberately looked for it. Essentially, they were never setting out to look for non-adult remains, but may have found them anyway and did not know how to interpret them.

Two subsequent influential articles, Sofaer Derevenski (1994) and Kamp (2001), both stressed how influential children can be on societal structures. Kamp (2001) specifically emphasized how children were involved with work and may have had a significant contribution in areas such as housework and agriculture. While household chores may not

seem significant, they help to alleviate work by the parents, and allow them to complete other necessary tasks. Children also require, however, energy from members of their community to survive (Kamp 2001). This is especially pertinent because this research focuses on a stage in life where children are particularly vulnerable. The energy limits of the sample's population may have influenced decisions regarding their nutritional intake.

Halcrow and Tayles (2011) have since outlined themes in childhood research that have occurred in sociology, anthropology, and archaeology, including social construction, agency, and play. Halcrow and Tayles (2011) establish that the biological immaturity of children is universal, but that the cultural definitions and modes of social transition into the adult world varies between groups. They also call attention to how caring for children requires energy from the adults, and this care will shape the way that individuals structure their lives. Halcrow and Tayles (2011) stress the importance of non-adults and what they say about morbidity and mortality. Halcrow and Tayles (2011) claim these ideas can been seen through the study of children because while the elderly individuals are supposed to die, when children die it is the result of "adverse environmental factors" (339).

Breastfeeding Biology

Breastfeeding is an important part of human life and has evolved with all mammals. Humans typically have a shorter weaning period than other animals, usually around 2.5 years, while chimpanzees will continue to 5 years old (Kennedy 2005). In general primates tend to mature over a longer period and as a result the milk is lower in protein and fat, which can be altered due to the quality of the mother's diet (Pike and Milligan 2010). Different cultures will

employ different weaning time periods. Breastfeeding frequency and schedule may also vary, indicating that some may breastfeed more times per day than others. Konner (2005) found that when comparing Western and non-Western feeding patterns that the former was more scheduled while non-western populations tended to breastfed continuously throughout the day.

An important concept to address is called the weanling's dilemma. This contemplates the decisions individuals must make regarding the energy required to continue breastfeeding and the health risks to the child that come with weaning. For example, great apes tend to invest more in their young and have high survival rates, however, population growth is slow (Kennedy 2005). Humans tend to have shorter weaning periods, which, while more dangerous for the young, increases the population (Kennedy 2005). Kennedy (2005) suggests that when populations wean earlier the survival of the infant is not the priority. Kennedy (2005) also emphasized the influence of human's social nature and that prolonged development allows for an increase in social skills necessary to live within the society. This emphasizes how important the environment and social aspects of the society influence on what may be considered a purely biological need.

Directly after birth, one of the benefits of a diet based only on breastmilk is that it provides immunity that is unique to the environment the infant was born into (Pike and Milligan 2010). Additionally, infants will not be exposed to any pathogens in the local water (Leung and Sauve 2005). The weaning period begins when the child starts consuming other foods in addition to breastmilk, and ends when they completely cease breastfeeding (Humphrey 2014). Breastfeeding exclusively for the first six months helps to reduce infant mortality, and after six months of age a child will need nutrients other than breastmilk (Kramer

and Kakuma 2002), as the nutritional and immunological constituents of breastmilk change (Kennedy 2005). Also, around 6 months of age the front deciduous teeth emerge, which some believe to mean the child is more ready for solid foods (Prowse et al. 2008).

As there are no written records from Meroe it cannot be known if there were recommendations for weaning foods. While Roman records indicate food such as animal milk, bread and cereals to create a pap, and eggs (Fildes 1986), it is not known if such practices of the Roman Empire influenced ancient Nubia. After the weaning process ends, children may still eat different foods than the adults in the population due to an immature digestive tract (Bogin 1997), or for cultural reasons (Humphrey 2010). In both contemporary and past populations, the decisions surrounding breastfeeding and weaning can be influenced by a variety of environmental and health risk factors, many that begin with the onset of the weaning process (McDade and Worthman 1998).

Humphrey (2014) discussed four stages of early life nutrition, including gestation, exclusive breastfeeding, weaning, and fully weaned. Table 2 shows nutritional pathways and how they correlate and overlap with the different stages (Humphrey 2014). These stages are important to understand for this research as these dietary transitions will impact individual nutritional access, and therefore can also impact stable isotope ratios recovered from bone tissues (Humphrey 2014).

Table 2 Early Nutrition Chart (derived from Humphrey 2014)

Stage 1: Gestation	Stage 2: Exclusive Breastfeeding	Stage 3: Weaning	Stage 4: Fully Weaned
• Nutrients from mother's digestive tract • Nutrients from placenta • Bone resorption increase from mother due to fetus's need for calcium	• Nutrients from mother's digestive tract • Nutrients from mammary gland • Nutrients from infant's digestive tract • Some energy from maternal fat and skeleton	• Nutrients from mother's digestive tract • Nutrients from mammary gland • Nutrients from infant's digestive tract • Some energy from maternal fat and skeleton • New foods are introduced to the infant	• Nutrients from infant's digestive tract • Diet may change as the child ages

In stage one (gestation) nutrients are taken from the mother (Cross et al. 1995; Krachler et al. 1999; Jay et al. 2008). In stage two (exclusive breastfeeding) the infant is still getting nutrients from the mother. The main difference is that nutrients are now transferred through the mammary gland (Krachler et al. 1999). Stage three (weaning) begins when food in addition to breastmilk is consumed, and stage four (fully weaned) is when the individual had finished breastfeeding completely (Katzenberg et al. 1996).

The ability for the mother to lactate is primarily the result of hormones that occur initially during pregnancy and continue as the child begins to breastfeed (Leung and Sauve

2005). When the infant begins to breastfeed, there is a complex balance between what they need and what the mother can offer. Moffat and Prowse (2018) note that this is not '"supply and demand'…[but] more accurately, 'demand and supply;"' (101).

In past populations it may have been the case that the infant would have been artificially fed, a decision based on biological and/or cultural factors such as maternal illness or death, economic need, or the desire to have more children (Fildes 1986). Interestingly, several child feeding cups have been recovered and identified from this particular cemetery (David, Pers. Comm.). While the exact use of these cups is not known, it has been suggested that they may have been used to supplement the diet, in cases of illness or if the infant's mother had died. Additionally, women of a higher economic status could hire wet nurses, as was done in Egypt during the same time period (Fildes 1986). This will be important to consider for the research because this population is considered "elite" and therefore may have earlier weaning periods than other groups in the same population, or may have had the resources to engage in alternative practices such as employing wet nurses.

Wet nurses are women who would breastfeed the child in place of their biological mother. There are instances in contemporaneous Roman Egypt of wet nurses being used (Dupras et al. 2001), and while there is no direct evidence for their use in Meroe, it demonstrates a possibility. If this practice existed in ancient Nubia, the elite members would most likely be the ones to use them. Oribasius, a Greek medical writer and physician in the fourth century, compiled dietary recommendations for the wet nurse from previous writers on infant health. Recommendations included bread, from barley and wheat, fish, meat and wine.

Foods to be avoided consisted of ones that tasted and smelt bad such as milk, garlic, and onion (Lascaratos and Poulakou-Rebelakou 2003).

Given the nature of the study population, it is also important to consider the Trivers-Willard hypothesis which postulates that in wealthier communities the female infants are weaned sooner, and in lower economic communities the male infants are weaned sooner. Wander and Mattison (2013), in their study conducted in Kilimanjaro, Tanzania, found that socio-economic status did indeed influence weaning timing between the sexes. They also found that there was a statistically significant correlation between weaning timing and birth order. The older children were weaned faster than their younger siblings. While sex cannot be estimated from non-adult skeletal remains, factors such as infant sex and birth order should still be considered as a potential interpretation for finding significant variation in isotope results.

Age Categories

This research is focused on non-adults, and it is important to note that age has different connotations depending on the context (Halcrow and Tayles 2011). As mentioned previously, scholars today recognize that childhood is a construct, and as a result, there is an acknowledgement of the nuances of different types of age categories (Halcrow and Tayles 2011), and incorporating social factors (Kamp 2001). It has also been argued that using developmental stages such as weaning (Wiley and Pike 1998), allows an understanding of the social side of a life (Halcrow and Tayles 2011).

There are three main age category types to consider: biological, chronological, and social. An individual's biological age deals with physiological functions pertaining to growth,

development, and eventually degeneration. An example of this is the estimation of age based on dental eruption (Halcrow and Tayles 2011). Chronological age is the actual time since the individual was born (Halcrow and Tayles 2011); however, it is important to recognize that some societies do not recognize chronological age (Fortes 1984). Social age is based on cultural behaviors and "status" that correlates to age groupings according to that society (Halcrow and Tayles 2011). It is important to recognize that an individual's biological, chronological, and social age can differ significantly. Each age type has its place within anthropological research.

Throughout the archaeological literature there are many words used to describe children, including sub-adults, non-adults, and juveniles (Halcrow and Tayles 2011). Throughout this research the term non-adult will be used to signify individuals under 17 years of age (Lewis 2007). Age categories within the non-adult category are nuanced (Halcrow and Tayles 2011; Lewis 2007). Although Baxter (2008) suggested that the creation of age categories such as "infant, toddler, child, adolescent, young adult, adult, and elderly" (161), have placed westernized aging norms into a place of standardization, placement into such categories is useful in understanding differences related to developmental growth. It is important to note, however, that age categories may be interpreted differently. For example, Lewis (2007) discusses how the "infant" category has been interpreted differently in studies, including definitions of under one year, and up to five years of age. For this study, specific age categories are developed to reflect growth and social development (Table 3). Halcrow and Tayles (2008) compared the age categories of non-adults from a variety of sources, which were considered when deciding the categories for this research.

Table 3 Biological Age Categories

Biological Age Category	Time Frame
Fetus	After 8 weeks in the uterus
Stillbirth	After 28 weeks in uterus and born dead
Neonate	Up to 27 days after the birth
Infant	Birth to one year
Young child	1-5 years old
Older child	6-14 years old
Adolescent	15-17 years old
Adult	> 17 years old

Derived from Lewis (2007) and Halcrow and Tayles (2008).

Halcrow and Tayles (2010) critique Lally and Ardren's (2008) discussion of biological and social age categories. Lally and Ardren (2008) argue that too much weight is put on biological age when examining infants. Halcrow and Tayles (2010) argue that while social responses are different, the immaturity and vulnerability of the infant are universal, and that the biological state of the infant could act as a constant while observing the cultural response, thereby indicating the larger societal structure. This is significant for the present research because breastfeeding can be a significant biological part of caring for an infant (Humphrey 2010). As a result, understanding this behavior in different communities may shed light on their social structures.

Stable Isotopes

The following section includes a discussion of the theory behind the main methodology to be used in this research, stable isotope analysis. Specifically, what stable isotopes are and the way this type of analysis applies to the proposed research. This method was chosen because it has been proven to provide direct evidence of the diet of prehistoric populations, which includes the process of weaning (Eerkens et al. 2018; Dupras et al. 2001).

Stable isotopes are the nonradioactive form of elements, which means they will not decay (Arnseth 2018). Isotopes have the same number of electrons and protons, but a different number of neutrons that affect the stability of the element (DeNiro 1987). The majority of elements have two or more stable variations (DeNiro 1987). The isotopic value will change as energy sources transfer between organisms due to a process called fractionation (Fujibagshi et al. 2016).

Isotopic fractionation is a natural process that occurs on an atomic and subatomic level (Fry 2006) resulting in a change to the state of the element, influenced by its weight (Arnseth 2018). Fractionation can occur in many different ways (Arnseth 2018). This research will focus on biochemical reactions, which tend to use lighter elements because it requires less energy (Arnseth 2018). As a result, animals tend to express heavier isotopic values (Fujibayashi et al. 2016). The main concern of fractionation for this research is understanding how much carbon and nitrogen change as they move through different organisms, and in particular what is expected to be found in humans.

Stable Carbon Isotopes

One of the elements that will be analyzed in this research is carbon. Stable carbon isotopes can be analyzed from bodily tissues to determine what types or group of foods were consumed by the study population. In particular it may shed light on the types of food non-adults in this population were eating during and after the weaning process. While in utero the fetus will have the same isotopic values as the mother, infants that exclusively breastfed will have tissues that show a slight enrichment of approximately 1‰ in their carbon isotope values due to a trophic level effect (or level of the food chain) (Fuller et al. 2006; Humphrey 2014).

The difference in ratios of $^{13}C/^{12}C$ is small and therefore an equation was developed by DeNiro (1987) to make the numbers easier to comprehend. The equation essentially takes a ratio of the light and heavy variation of the element and compares it to the ratio of a standard (or material of known value) (Arnseth 2018). The value, expressed in the measurement unit permil (‰) is represented as δ value or delta value (Arnseth 2018). For carbon, the notation is $\delta^{13}C$ (DeNiro 1987). The equation developed by DeNiro (1987) is:

$$\frac{\frac{Amount\ of\ ^{13}C\ in\ Sample}{Amount\ of\ ^{12}C\ in\ Sample}}{\frac{Amount\ of\ ^{13}C\ in\ Standard}{Amount\ of\ ^{12}C\ in\ Standard}} - 1 \times 1000\% \qquad (1)$$

The standard to which DeNiro (1987) compared carbon values was derived from the calcium carbonate of a fossil belemnite found in South Carolina (DeNiro 1987), and as such, carbon isotope values were determined by a PeeDee Belemnite (PDB) scale (DeNiro 1987). The standard for this research is Vienna PeeDee Belemnite (VPDB). The different ratios of carbon values in terrestrial plants are the result of multiple variables (DeNiro 1987). This includes the makeup of the atmospheric CO_2, where the plants get their carbon, and the fractionation that occurs as the CO_2 becomes carbon in the plant (DeNiro 1987).

Different plants will have variations of $\delta^{13}C$ that are reflected in the carbon of its consumer. Plants can be divided into three main categories based on their ability to fix atmospheric CO_2 (Figure 2). These three categories are C_3, C_4, and CAM plants, all differentiated by the number of carbon atoms that are formed during the fixation of atmospheric CO_2 (DeNiro 1987). This difference is specifically linked to the enzyme that each plant uses for CO_2 fixation. C_3 plants use ribulose bisphosphate carboxylase and C_4 plants use

phosphoenol pyruvate carboxylase (DeNiro 1987). Additionally, crassulacean acid metabolism (CAM) plants can use either the C_3 or C_4 photosynthetic pathway, dependent on the local environment (DeNiro 1987). It is also important to consider that the atmospheric levels of CO_2 changed after the industrial revolution because of the use of fossil fuels (DeNiro 1987), therefore affecting carbon isotope values. It is also valuable to remember that the seasons and yearly changes in weather may impact the variation in values between the past and present (DeNiro and Epstein 1978).

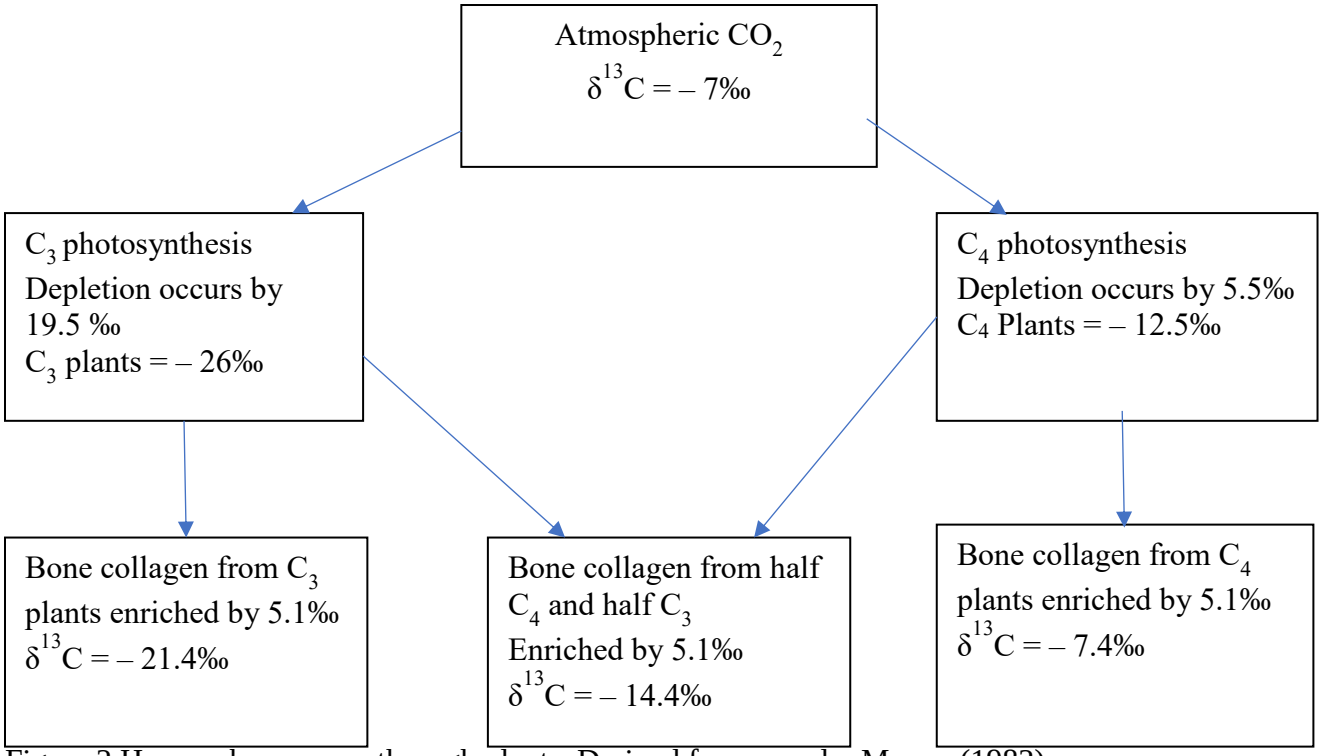

Figure 2 How carbon moves through plants. Derived from van der Merwe (1982).

Plants are important to understand because they are at the base of the food chain and therefore influence the isotopic values of their consumers (DeNiro 1987). C_3 plants typically have values that fall around –26‰ (DeNiro 1987). Some foods in this group include rice,

beans, tubers, nuts, and most trees, shrubs, and vegetables. Additionally, honey normally has a carbon value reflecting the C_3 plants from which bees gather pollen (Doner and White 1977). C_4 plants typically have values that fall around –11.5‰ (DeNiro 1987). Some foods in this group include maize, teosinte, amaranth, sugar cane, sorghum, some millets, and most xeric grasses (DeNiro 1987). CAM plants include plants such as agave, yucca, pineapple, and prickly pear (DeNiro 1987). It is also important to recognize populations may have a diet that contains both C_4 and C_3 plants.

Carbon occurs in both terrestrial and marine life, which means it is also important to understand the marine carbon cycle. An important difference between the carbon cycle of terrestrial versus marine plants is that marine plants use bicarbonate suspended in the ocean water, while terrestrial plants use CO_2 from the atmosphere (DeNiro 1987). Richards and Hedges (1999) stated that carbon in marine C_3 plants is 8‰ heavier than those in terrestrial environments. They attributed this to the fact that C_3 plants in marine environments experiences less fractionation.

Given that the population under study lived on the Nile River, it reasonable to assume they may have eaten fish. These fish, however, would be freshwater fish, and most likely not marine based organisms. Fish consumption in archaeological context has not always be accurately represented, which is attributed to cultural practices that do not leave bones behind in excavation sites (Katzenberg 2008). Additionally, if specific excavation methods are not used, such as “fine screening”, relevant remains may not be collected (Katzenberg 2008: 426). As a result, the specific fish being eaten by the population may not be known, which can make interpretation difficult.

There is more variation in $\delta^{13}C$ in freshwater fish than terrestrial organisms due to many different sources of carbon (Katzenberg 2008). These sources include the atmosphere, the water, rocks and soil, and waste from the other plants and animals in the ecosystem (Zohary et al. 1994). The depth of the water has also been shown to impact the carbon values, with shallow waters presenting higher $\delta^{13}C$ ratios (Katzenberg and Weber 1999). While stable isotope studies of freshwater fish show great variation in $\delta^{13}C$ values, their values more greatly resemble those of C_3 plants. For example, Oczkowski et al. (2008) found that fish from the Nile Delta in Egypt including tilapia, mullet, carp, and catfish, have $\delta^{13}C$ values that range from – 20‰ to – 30‰. Although analysis of fish bones from Sai Island is not possible, we can assume that the $\delta^{13}C$ values would likely be similar to C_3 plants.

Knowing what foods were typically eaten is important when interpreting the amount of C_4 and C_3 foods eaten in the diet. In Nubia C_3 plants were shown to be of more importance, but during the Meroitic period C_4 plants such as sorghum and millet became more prevalent (Rowley-Conway 1989). Also, C_4 grasses could have been part of the diets that the animals were consuming (Iacumin et al. 1998). The Meroitic elite may have eaten more wheat and barley, which were traditional Meroitic foods while the rest of the population would have eaten more millet-based foods (Haaland 2014). Known foods can be seen in Table 4.

Table 4 Potential foods and their carbon group (Derived from Edwards (2004), DeNiro (1987), Fisher (2012), Haaland (2012; 2014), Iacumin et al. (1998), Ikram (2012), and Oczkowski et al. (2008))

	Grains and Seeds	**Fruit**	**Vegetables and Legumes**	**Animals***
C_4 values	Sorghum *(Sorghum bicolor)* Pearl millet *(Pennisetum glaucum)*			Cattle *(Bos Taurus)* Sheep *(Ovis aries)* Goats *(Capra aegagrus hircus)*
C_3 values	Barley *(Hordeum vulgare)* Wheat *(Triticum)* Sesame Seeds *(Sesamum indicum)*	Grapes *(Vitis)* Melon *(Cucurbitaceae)* Olives *(Olea europaea)*	Beans *(Phaseolus vulgaris)* Peas *(Pisum sativum)* Pepper *(Capsicum)*	Fish Cattle *(Bos Taurus)* Sheep *(Ovis aries)* Goats *(Capra aegagrus hircus)*

*Animals are listed as both C_3 and C_4 due to the possibility of being fed both C_3 and C_4 grasses and grains.

As there is limited information about children in the Meroitic period it is not possible to cite known weaning foods. Information from a contemporaneous and somewhat geographically close site can be used as an inference. Dupras et al. (2001) in sample from a Roman Egyptian site demonstrated evidence of C_4 fed cow/goat milk and/or C_4 based pap being used for

weaning. When combined with the foods listed in Table 4, potential C_3 weaning foods would include barley and wheat pap, and the milk of animals that fed on C_3 grasses, and examples of C_4 weaning foods would include sorghum and millet pap and the milk of animals that fed on C_4 grasses. Given the cyclical and seasonal nature of the grain crops, infants may be eating one or the other, or in some cases both. In addition, it is possible that infants would eat a mixture of C_3 and C_4 weaning foods if the animals ate foods with mixed carbon values such as barley and millet.

Stable Nitrogen Isotopes

Stable nitrogen isotopes are commonly used to understand an organism's place in the food chain due to the trophic level effect (Katzenberg and Pfeiffer 1995). If an animal is higher up in the food chain, its nitrogen value will be higher than the modeled environmental baseline. For example, plants that get nitrogen from decomposing matter in the soil are going to have higher values than ones that do not (Katzenberg and Pfeiffer 1995). It should be noted that there are differences in the nitrogen values of prehistoric plants compared to contemporary plants due to the modern use of chemical fertilizers (DeNiro 1987).

Stable nitrogen isotopes are expressed in the notation $\delta^{15}N$ (DeNiro 1987), and their standard until of measure is permil (‰). To understand the amount of stable isotopes present in a sample, the difference in a ratio of $^{15}N/^{14}N$ is represented in an equation developed by DeNiro (1987) that aims to amplify the subtle differences. The standard against which samples are measured is AIR (atmospheric N_2). The equation for $\delta^{15}N‰$, developed by DeNiro (1987) is:

$$\frac{\frac{Amount\ of\ 15\text{N in Sample}}{Amount\ of\ 14\text{N in Sample}}}{\frac{Amount\ of\ 15N\ in\ Standard}{Amount\ of\ 14N\ in\ Standard}} - 1 \times 1000\% \qquad (2)$$

How the environment can potentially impact nitrogen isotope values is important to understand when making interpretations of stable nitrogen isotope values. For the samples used in this research, it may be expected to see somewhat of an increase in nitrogen values because of the arid environment. There are two potential causes of stable nitrogen isotope variation in arid environments including animal physiological adaptation and desert soil nitrogen availability. In the case of animal physiology, water conservation (Ambrose and DeNiro 1986) and recycling gut material (Sealy et al. 1987) can impact stable nitrogen values by increasing nitrogen (Thompson et al. 2008). In time of water stress more urea waste is excreted, which causes a loss of ^{14}N, and increases the δ^{15}N values (DeNiro 1987). Secondly, it has been demonstrated that, overall, the entire food chain in desert environments have higher stable nitrogen isotope values (e.g., Ma et al. 2012). Schwarcz et al. (1999) postulate that higher ^{15}N in desert soils may be the result of the evaporation of isotopically light ammonia formed as the result of bacterial activity. This increase in desert soil δ^{15}N is then passed on to the consumers and through the food chain, resulting in significantly higher δ^{15}N values.

Stable nitrogen isotopes can be used to determine the timing of infant breastfeeding and weaning processes. The seminal article by Fogel et al. (1989) demonstrated that the keratin in fingernails of breastfeeding infants had increased nitrogen compared to their mothers. A breastfeeding infant will have a nitrogen isotope value that is 2-3‰ higher than their mother (Katzenberg and Pfeiffer 1995) because the infants are technically higher on the food chain as consumers of their mother's nutrients. Nitrogen isotope values will decrease as the infant starts

to be weaned and becomes more reliant on supplementary foods. When breastfeeding is finished, the infant's or child's nitrogen isotope values will reflect the food it ingests and should eventually be similar to the mother's if eating a similar diet (Fuller et al. 2006). It is also important to consider the types of foods used for weaning. If infants are being weaned on foods with higher nitrogen values, the results may be misleading.

Another consideration to make during the interpretation of the data from this study is the fact that growing children will have a metabolic make-up distinct from an adult who has reached a point of stability (Schwarcz 2000). This is important to consider when interpreting nitrogen isotope values because the infant requires higher nitrogen intake for the creation of cells, and for the mineralization process in bone (Schwarcz 2000). Fuller et al. (2006) suggested that when children are breastfed there may be less of a trophic level shift shown because of their rapid growth. When an individual is experiencing rapid growth, their body may select the heavier ^{15}N instead of the lighter ^{14}N, due to efficiency, (Hare et al. 1991; Kendall and Caldwell 1998). Bone collagen addition, however, is slower than other proteins, like those directed at muscles, so protein for bone addition may have time more selective of nitrogen isotopes (Waters-Rist and Katzenberg 2010). Waters-Rist and Katzenberg (2010) suggest that more infant isotopic studies are needed to understand how high bone turnover rate associated with infant growth impacts stable nitrogen isotopes values.

Other considerations concerning the interpretation of nitrogen isotope values includes nutritional stress, and how in some cases certain health conditions can cause enrichment in $\delta^{15}N$ values. Katzenberg and Lovell (1999) examined how certain health conditions can impact nitrogen isotope ratios, and found that while bone, due to slow turnover, should not be affected

as much as other tissues, nutritional stress can produce nitrogen isotope value enrichment. Reitsema (2013), while noting that poor nutrition may not readily appear in bone isotope ratios, advocated for using stable isotope analysis to track disease in the living and past due to the notable differences in nitrogen isotope values found in sick individuals. Olsen et al. (2014) examined collagen from individuals with known pathological conditions and compared the variation in isotopic signatures against a normal sample population. It was found that when the bone sample was taken near a lesion that isotope values would increase. Additionally, Beaumont et al. (2015) have demonstrated that $\delta^{15}N$ values may be elevated if individuals had poor nutrition. These factors are important to consider when interpreting the isotopic values from the present research sample, given that the sample consists of non-adults who died in childhood.

Stable Isotopes and Weaning Studies

Many previous studies have used stable isotopes to research weaning patterns. In one of the earliest published examples, Katzenberg and Pfeiffer (1995) examined 77 individuals from an upper-class historic cemetery in Ontario, Canada. Stable nitrogen isotope values, in addition to historical demographic information, were used to interpret the weaning process. One key cultural pattern Katzenberg and Pfeiffer (1995) derived from historical records was that wealthier women weaned their infants earlier than rural women because the wealthier women had access to wet nurses. This study demonstrates how understanding the healthcare of a population may be useful in interpreting the estimated weaning times. Katzenberg and Pfeiffer (1995) estimated through stable isotope analysis that weaning happened a few months before the infant turned one, correlating with the historic information available, and supporting the

accuracy of their methodology. The idea of status and weaning patterns will be considered in this research.

Another example of such a study concerned the estimated time of weaning in a population from the Romano-Christian period at Kellis in Egypt (Dupras and Tocheri 2007). The authors used a longitudinal analysis of infant and non-infant stable isotope data to estimate dietary patterns. The study utilized the nitrogen, carbon, and oxygen isotopes of deciduous and permanent dentition from 27 juveniles and 75 adults. Teeth form at distinct times, and do not remodel, and can be used to determine how diet changed throughout an individual's life. Dupras and Tocheri (2007) found that infants were breastfed exclusively until six months of age and then C_4 foods were introduced to their diet, and that the weaning process lasted until three years of age. These results supported an earlier study conducted by Dupras et al. (2001).

A third example is the examination of weaning patterns and juvenile health in medieval Japan using carbon and nitrogen stable isotope analysis of the bone collagen from and 58 non-adults and compared against previously reported adult data (Tsutaya et al. 2015). This study was intended to add to previous research of the site, which identified higher stress and mortality rates in the non-adults in the population. The authors found little historical information available about recommended weaning age in medieval Japan. While significant variation in weaning age was noted, Tsutaya et al. (2015) found that the weaning process typically ended around three years of age. The variation found in age was attributed to cultural differences in treatment of the different sexes. Additionally, historical records indicated that there was food stress due to war, famine, and isolation. Tsutaya et al. (2015) attributed a long weaning period and lower birth rate to postpartum amenorrhea caused by this food shortage.

Previous research on breastfeeding patterns based on the same Meroitic population as used for the present study, used the first molars of 11 adults and determined the average weaning age to be 2.7 years of age (Eerkens et al. 2018). A difference in weaning patterns was found between the sexes, with males being weaned earlier. Most males were weaned on average by 2.4. Two of the females had higher ages of weaning (3.9 and 3.0). The small sample size means that these results should not be generalized across the population, and the authors recognized the need for further studies, but note gender differences should be considered. In addition, it should be noted that those individuals survived to adulthood, and the individuals in this study did not. This leads to the possibility of different weaning patterns in individuals who died young and those that survived to adulthood.

The study by Katzenberg and Pfeiffer (1995) demonstrates the accuracy of the isotopic method as it was compared against historical records. Additionally, it addresses considerations when examining an elite population. The study by Dupras and Tocheri (2007) is focused on a site that is geographically and temporally close to the sample population and Tsutaya et al. (2015) addresses how stress can impact weaning patterns. Lastly, previous research from the same population was reported by Eerkens et al. (2018). Many other studies will be considered when analyzing data. Some comparison studies from a similar time period and cultural influence to the sample population can be seen in Table 5.

Table 5 Weaning comparison studies

Time Period and location	Material	Estimation of weaning	Considerations	Citation
Roman and Late Roman Leptiminus, Tunisia. 4 cemeteries between 2nd and 6th centuries AD	Bone collagen Nitrogen and carbon isotopes	Began before the age of 2 and ended by the age of 3 Historical records show recommendation of weaning by 6 months	Wet nurse contracts Diet of mother's recently gave birth were different from other adult females	Keenleyside et al. (2009)
Roman Oxfordshire, UK 3 Cemeteries from between 1st and 6th centuries AD	Bone collagen Nitrogen carbon and sulfur Isotopes	Weaning by complete by 3-4 years old Weaning foods consisted of freshwater protein (riverine fish or cereal) Older child had no evidence of freshwater protein	Sulfur was able to identify more variation in diet including freshwater protein Flooding considerations	Nehlich et al. (2011)
Byzantine Era Greece 6th and 15th centuries AD	Bone collagen Nitrogen and carbon isotopes	Weaned before the age of 4 Some had elevated $\delta^{15}N$ at 3 years old Found a lot of variation	Noted an increase in non-adult mortality as $\delta^{13}C$ and $\delta^{15}N$ lower during ages 2-3. Suggested this may be due to insufficient or contaminated weaning food.	Bourbou et al. (2013)

Bone Collagen

All biological tissues can be used for stable isotopes analysis and they all have their advantages and disadvantages. Bioarchaeological analysis normally focuses on bone and teeth, however other tissues such as hair, fingernails, and skin have been used. When using bone, both collagen and/or apatite can be extracted for analysis, and represent different portions of the diet. The biological tissue chosen for this analysis is bone collagen. Collagen is a protein molecule that makes the bone slightly flexible and helps prevent breaks (White et al. 2012), and it is the most common protein in the body, making up about 90% of the organic part of adult bone (White et al. 2012). Collagen was chosen because it specifically reflects the carbon from dietary protein whereas apatite reflects carbon from the entire diet including lipids, carbohydrates, and protein (Williams et al. 2005). For this study collagen is particularly important as it contains a nitrogen component. This will enable the analysis to control more for what specific factors are influencing the isotopic values because the combination of carbon and nitrogen isotopes from collagen allows for a focus on both protein and trophic level.

In bioarchaeological research, collagen preservation can be an issue. Collagen is protected by hydroxyapatite, which is insoluble (Grupe et al. 2000). However, soil, particularly those that are acidic, can still alter the protein structures, and specifically the amino acids. Microbial degradation is the main cause of alterations in collagen. Fungi and bacteria within the soil can use proteins, like collagen, for their nutrients and such metabolic action can impact the stable isotope signature (Grupe et al. 2000). Due to this phenomenon, particular attention is given to the determination of sample preservation, which will be discussed in Chapter 4.

CHAPTER THREE: MATERIALS AND METHODS

This section includes details of the materials and methodology used for analysis. This includes the presentation of age estimation, how the collagen was extracted from the samples, and statistics. A total of 50 non-adult samples and 50 adult samples, which includes duplicate samples, will be analyzed for stable carbon and nitrogen isotopes of bone collagen. For all samples the femoral bone was used for analysis.

Age Estimation

Age for the non-adult skeletal remains were estimated in the field by Dr. Tosha Dupras using dental development methods Liversidge and Molleson 2004; Liversidge et al. 1998; Moorrees et al. 1963a and b; Smith, 1991; Ubelaker 1978), long bone length aging methods (Anderson et al. 1964; Fazekas and Kosa, 1978; Maresh, 1970; Scheuer et al. 1980), and epiphyseal development and fusion aging methods (Haines et al., 1967; McKern and Stewart, 1957). It should be noted that these are estimates, which have inherent error, and this should be considered when analyzing the data.

Bone Collagen Extraction

The methodology for this research is stable nitrogen and stable carbon isotope analyses of collagen extracted from bone samples. The protocol that was used follows that of the UCF Bioarcheology Lab Stable Isotope Tech Memo # 01-02, adapted from Longin (1971). The first step was to clean and grind the bone samples. The samples were then washed in distilled water using an ultrasonic bath, which uses sonic vibrations to remove dirt and soft tissue still adhered

to the bone. The samples stayed in the sonicator for 10-minute intervals, the water was replaced each time until the water remained clear. After washing, the samples were placed in the oven to dry at 60°C for 24 hours. After the bones were cleaned, a total sample of 1 to 3 grams was removed. The sample was then ground down to fragments of approximately 5mm in size and placed in a 50ml centrifuge tube.

The next step was to remove lipids. This step is not always necessary for archaeological samples as lipids are not typically found in dry ancient bones (Bocherens et al. 1996). This step is advised, however, in samples that may be well preserved such as those from mummified corpses. In the case of the samples from Sai Island, soft tissue preservation was evident, and as such, this step was followed. This particular method uses a mixture of 2:1 chloroform: methanol. Using a pipette, 10 ml of the 2:1 chloroform: methanol solution was added to the sample, and the sample was then agitated and left for 20 minutes with the cap untightened. The samples were then spun down in the centrifuge for 10 minutes. The solution was decanted using the pipette and then this step was repeated two more times. If the solution changed color, or there were lipids on the surface, the liquid was decanted and the rinse was repeated until the liquid remained clear. After the chloroform: methanol solution was removed for the last time, the samples were left to dry for 24 hours in the fume hood.

The next step involved the demineralization of the bone. This step used a weak solution of hydrochloric acid (HCl) to remove the inorganic components of the bone. For this procedure, 10 ml 0.25 M HCl was added to each sample. The sample was also agitated occasionally on an oscillator to speed up the reaction. This is based on the principal that movement will speed up a chemical reaction. Each day the pH of the sample was tested, and if

a pH level of more than 1.0 was noted, the acid was decanted after centrifuging and replaced with new HCl. The samples were determined to be demineralized when they became softer and more flexible with an almost gel-like consistency.

After the samples were demineralized, the final HCl solution was decanted and then 10ml of distilled water was added to the sample. The sample was then spun down in the centrifuge for 10 minutes, decanted, and this process was repeated for a total of three times. If the desired pH, 2.5 and 3.0, was not reached at this point, the rinses were repeated until the desired pH was achieved.

The next step was to remove the humic acids. These are parts of the soil that could still be in the sample and can impact the isotope makeup. Since they are soluble in bases, the protocol uses sodium hydroxide, or NaOH. There are some studies (e.g. Ambrose and DeNiro 1986), which use treatments that last almost a day, but this protocol used a shorter time length because it has been shown to have less of an impact on the collagen (DeNiro and Epstein 1981; Katzenberg 1989). Approximately 10 ml of 0.1M NaOH solution was added to each sample and left for 20 minutes. If a color change was observed (potentially ranging from light yellow to black depending on the amount of humic acids present) then the NaOH was decanted and new NaOH was added for another 20 minutes. These steps were repeated until there was no observed color change.

After the NaOH treatment, the liquid was decanted, and the sample was rinsed with 10ml of distilled water for a minimum of six times (centrifuging and decanting in between each rinse). After the sixth rinse, the expected pH is 7, and if not, the rinses were repeated, or a buffer was added to reach the desired pH level. For these samples more rinses were needed and

eventually it was decided to use the buffer to gain the pH level. After the sample reached the proper pH the water was decanted, 10 ml of HCL was added, and then spun down. After the acid was decanted, 5 ml of distilled water was added to each sample, yielding a pH level of 2.5 to 3. The samples were capped loosely and then placed in the oven at 60°C for approximately 16 hours.

Samples were then removed from the oven and spun down in the centrifuge for 10 minutes. At this stage, the collagen was water soluble. The liquid was then transferred to a pre-weighed 3-dram vial. Any residue was left at the bottom. The vials were then placed in the oven at 90°C, uncapped, until they were dry, approximately 24-36 hours. The vials were then weighed again. The last step before sending for analysis was to calculate the collagen yield using the formula:

$$\%\text{Collagen Yield} = \frac{Collagen\ weight\ (g)}{Sample\ dry\ weight\ (g)} \times 100 \qquad (3)$$

Samples were then sent to the Department of Geological Sciences at the University of Florida for the final analysis on an isotope ratio mass spectrometer. The Mass spectrometer used was the Finnigan-MAT DeltaPlus isotope ratio mass spectrometer with a ConFlo II interface attached to a Carlo Erba elemental analyzer.

Statistical Analysis

Due to limited sample size, particularly in each age category, statistics can only be used in a narrow way. Normally, when comparing the mean values from two different groups of individuals a Student's Independent T-test would be used. There are several assumptions, however, that must be met when using a T-test, including a normal distribution. Given the size

of demographic categories used in this research, a normal distribution cannot be assumed. There are a number of nonparametric tests that do not require assumptions including the Mann-Whitney U test. Smaller sample sizes and abnormally distributed samples can be compared using this test. The calculation will be using a two-tailed hypothesis and the level of significance will be .05.

CHAPTER FOUR: RESULTS

This chapter includes an examination of the isotopic data, which includes a consideration of sample preservation, and the results of the stable carbon and nitrogen isotope analyses. Sample preservation will be determined through the results of the percent collagen yield, the atomic C:N ratios, and the weights of % carbon and nitrogen. The isotope results pertinent to the research question regarding weaning patterns will then be presented through a series of graphs, which compare different demographics of the population. Due to the very small samples sizes in some age categories, standard statistical tests could not be used widely, as a result trends in the data, represented by graphs, will be the main focus. When possible, the Whitney Mann U statistic will be reported.

Data Precision and Accuracy

The samples for this thesis were run on three separate days. Machine precision based on laboratory standards are reported as follows: Day one: $\delta^{13}C = \pm 0.10‰$ and $\delta^{15}N = \pm 0.13‰$ (N=5), Day two: $\delta^{13}C = \pm 0.05‰$ and $\delta^{15}N = \pm 0.07‰$ (N=7), and Day three: $\delta^{13}C = \pm 0.02‰$ and $\delta^{15}N = \pm 0.18‰$ (N=7). In addition, 10 sample duplicates were analyzed to determine the accuracy of the results. The difference between the replicates were calculated and averaged (Table 6). For the interpretation of this data, the average value of the duplicates will be used. Overall, the accuracy of the sample analysis, with the exception of the $\delta^{13}C$ values of To-042 Ind 2, To-043, and Te-013, were close, specifically below 1, suggesting that the values for the rest

of the samples can be trusted as accurate. Those samples with large differences are not included in the analysis due to preservation standards discussed in the next section.

Table 6 Accuracy Calculation for Duplicate Samples

Sample ID	Differences in $\delta^{15}N‰$	Differences in $\delta^{13}C‰$
To-040	0.07	0.04
To-042 Ind 1	0.35	0.70
To-042 Ind 2	0.33	2.23
To-043	0.30	2.94
Te-004 Ind 1	0.29	0.48
Te-007/008 Ind 1	0.52	0.22
Te-013	0.96	1.93
Te-014 Ind B	0.13	0.12
Te-015 (2)	0.09	0.11
Accuracy	**.34**	**.97**

Sample Preservation

As previously discussed in Chapter Two, collagen can degrade over time and be impacted by the environment, and it is important to be able to discern a sample's preservation in order to trust the reliability of the isotope data. The preservation of the samples was judged based on three criteria, percent collagen yield, the % weight of the nitrogen and carbon, and atomic C:N ratio. Table 7 shows the data that will be used to determine the preservation of the original 100 samples that were analyzed.

Table 7 Preservation Data for the Elite Meroitic Samples from 8-B-5.A

Original Sample ID	%Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	atomic ratio C:N
To-001 Ind 5	8.50	11.55	-17.34	15.59	41.68	2.67	3.12
To-004 Ind 1	0.37	11.66	-17.20	12.45	34.2	2.75	3.20
To-004 Ind 2	2.98	12.35	-15.94	15.4	42.19	2.74	3.20
To-005 Ind 1	12.85	11.50	-17.53	15.84	43.74	2.76	3.22
To-005 Ind 2	13.46	12.21	-16.57	16.13	44.07	2.73	3.19
To-005 Ind 3	7.36	11.87	-15.40	15.50	41.98	2.71	3.16
To-017 Ind 2	14.13	11.15	-16.72	15.06	40.65	2.70	3.15
To-017 Ind 3	11.89	12.34	-16.67	16.18	43.68	2.70	3.15
To-017 Ind 4	10.32	12.26	-13.84	15.44	42.37	2.74	3.20
To-017 Ind 5	7.78	11.13	-17.33	12.61	35.01	2.78	3.24
To-018 Ind 1	8.23	11.42	-13.67	15.41	42.37	2.75	3.21
To-018 Ind 2	16.42	10.81	-17.92	16.76	45.06	2.69	3.14
To-018 Ind 3	6.93	11.94	-17.67	15.12	41.87	2.77	3.23
To-018 Ind 4	12.68	11.23	-18.76	15.82	43.59	2.76	3.21
To-024	2.14	13.92	-14.49	3.69	11.49	3.11	3.63
To-024	0.33	13.65	-16.76	0.81	9.67	11.96	13.96
To-026A	0.178	11.82	-17.31	6.42	24.09	3.76	4.38
To-026B	4.87	11.35	-16.82	13.92	38.51	2.77	3.23
To-026C-2	1.6	13.12	-17.26	4.32	13.42	3.11	3.62
To-027	0.422	14.57	-14.71	8.06	26.27	3.26	3.80
To-027 Ind 1	4.76	11.28	-17.36	15.79	43.36	2.75	3.20
To-027 Ind 2	2.28	11.12	-16.97	14.41	39.39	2.73	3.19
To-027 Ind 3	2.91	8.62	-16.43	15.71	43.01	2.74	3.19
To-027 Ind 4	1.47	11.50	-15.98	6.06	17.01	2.81	3.27
To-027 Ind 5	4.63	13.65	-14.82	6.26	17.55	2.80	3.27
To-027 Ind 6	2.29	12.22	-16.11	6.98	19.79	2.84	3.31
To-028 Ind 1	10.839	12.13	-15.30	14.13	40.22	2.85	3.32
To-028 Ind 2	8.965	14.59	-14.80	14.92	41.35	2.77	3.23
To-029	0.27	13.30	-14.93	12.47	35.73	2.87	3.34
To-029A	1.954	14.02	-13.78	12.64	35.40	2.80	3.27
To-030 Ind 1	4.42	11.47	-16.95	15.1	41.22	2.73	3.18
To-030 Ind 3	0.67	13.61	-13.25	12.53	34.28	2.74	3.19
To-031 Ind A	6.24	9.83	-17.07	14.67	41.14	2.80	3.27
To-031 Ind B	2.66	8.70	-18.11	4.74	14.17	2.99	3.49

Original Sample ID	%Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	atomic ratio C:N
To-031 Ind C	2.68	9.66	-17.11	11.12	31.29	2.81	3.28
To-034A Ind 1	9.301	11.48	-15.82	10.80	31.41	2.91	3.39
To-034A Ind 2	9.63	11.10	-17.51	15.27	42.89	2.81	3.28
To-034A Ind 3	10.351	10.83	-17.08	15.24	42.20	2.77	3.23
To-035 Ind 1	9.957	11.77	-16.63	15.40	43.18	2.80	3.27
To-035 Ind 2	10.124	12.99	-15.20	14.85	41.37	2.79	3.25
To-036 Ind 1	2.6	13.21	-14.92	13.03	35.61	2.73	3.19
To-038 Ind 1	0.91	13.24	-15.51	12.61	34.76	2.76	3.22
To-038 Ind 2	5.42	11.66	-15.60	14.61	39.59	2.71	3.16
To-040	6.38	11.64	-12.64	13.58	39.17	2.88	3.36
To-040	6.38	11.57	-12.68	13.81	39.86	2.89	3.37
To-040 Ind 3	10.02	10.86	-16.45	15.20	41.48	2.73	3.18
To-041	8.1	11.37	-17.51	15.64	42.85	2.74	3.20
To-042 Ind 1A	7.27	10.88	-15.28	13.94	41.51	2.98	3.47
To-042 Ind 1B	10.99	11.23	-15.98	13.34	39.21	2.94	3.43
To-042 Ind 2A	9.86	11.28	-15.35	14.95	41.89	2.80	3.27
To-042 Ind 2B	11.6	11.61	-17.58	15.75	43.60	2.77	3.23
To-043	9.64	11.69	-18.05	15.72	42.75	2.72	3.17
To-043 Ind 1	13.17	11.99	-15.71	15.11	42.62	2.82	3.29
To-043 Ind 2	10.36	12.12	-16.66	14.24	42.15	2.96	3.45
To-049 Ind 1	0.93	15.25	-14.75	4.89	21.95	4.48	5.23
To-049 Ind 2A	0.71	21.57	-14.93	0.62	4.40	7.06	8.24
To-049 Ind 2B	0.39	21.72	-14.73	0.98	6.17	6.33	7.38
To-049 Ind 3A	2.7	18.90	-15.76	1.05	7.25	6.93	8.08
To-049 Ind 3C	0.35	N/A	N/A	N/A	N/A	N/A	N/A
To-049 Ind 3D	0.12	12.61	-20.44	2.26	19.63	8.68	10.12
To-049 Ind 3G	0.82	17.15	-17.50	0.44	3.36	7.67	8.95
To-049 Ind 3H	0.32	17.91	-15.80	1.25	10.13	8.08	9.42
To-054 Ind 1	5.93	11.82	-16.38	13.99	39.03	2.96	3.45
Te-001 Ind A	0.87	14.79	-17.87	11.17	30.94	2.77	3.23
Te-001 Ind B	3.3	14.39	-18.13	8.39	22.16	2.64	3.08
Te-002 Ind 1A	0.35	11.58	-20.71	1.68	4.36	2.60	3.03
Te-002 Ind 1B	0.71	13.47	-20.26	1.10	4.10	3.72	4.34
Te-002 Ind 2	0.53	12.91	-16.91	2.26	4.33	1.92	2.24
Te-002 Ind 3	2.97	11.70	-16.19	13.41	35.97	2.68	3.13
Te-003	0.56	13.33	-15.29	2.21	6.08	2.75	3.21

Original Sample ID	%Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	atomic ratio C:N
Te-003 Ind 2A	0.1	12.50	-20.74	1.87	8.17	4.36	5.08
Te-003 Ind 2B	1.09	10.94	-17.70	0.87	3.14	3.59	4.19
Te-004 Ind 1A	2.82	14.91	-16.82	10.81	30.23	2.80	3.26
Te-004 Ind 1B	0.26	14.62	-17.30	8.45	22.78	2.70	3.15
Te-004 Ind 2A	6.67	12.53	-16.24	9.71	32.48	3.34	3.90
Te-004 Ind 2B	1.15	12.47	-16.74	5.52	15.01	2.72	3.17
Te-004 Ind 3	4.95	11.13	-17.61	13.59	37.38	2.75	3.21
Te-005	2.18	8.90	-17.49	12.87	35.44	2.75	3.21
Te-007/008 Ind 1A	6.35	13.75	-15.01	15.80	43.76	2.77	3.23
Te-007/008 Ind 1B	2.65	13.23	-15.23	6.00	17.25	2.87	3.35
Te-011 Ind A	3.1	11.26	-18.22	12.91	35.87	2.78	3.24
Te-011 Ind B	0.02	12.13	-18.20	7.15	23.38	3.27	3.81
Te-013 (a)	1.11	13.28	-16.53	11.98	33.24	2.77	3.24
Te-013(b)	1.04	14.24	-14.60	2.48	6.92	2.79	3.26
Te-014a	1.1	14.25	-16.47	4.60	12.64	2.75	3.21
Te-014 Ind B	1.05	14.38	-16.59	1.71	5.11	3.00	3.49
Te-015 (2a)	4.64	12.09	-15.07	10.91	29.98	2.75	3.20
Te-015 (2b)	4.34	12.00	-14.96	11.45	31.67	2.76	3.23
Te-017a	6.52	13.76	-14.88	14.52	41.54	2.86	3.34
Te-017b	9.55	13.38	-15.91	13.18	36.46	2.77	3.23
Te-019	7.69	12.05	-16.83	15.42	42.54	2.76	3.22
Te-026	0.28	11.30	-15.92	15.07	45.17	3.00	3.50
Te-033	2.26	10.62	-17.22	14.34	39.86	2.78	3.24
Te-034	3.13	11.50	-16.32	14.49	39.9	2.75	3.21
Te-035	0.45	10.94	-18.29	5.87	20.46	3.49	4.07
Te-057	0.44	11.34	-15.42	15.16	42.26	2.79	3.25
Te-063	2.12	10.29	-18.67	11.48	32.47	2.83	3.30
Te-066 IND A	6.23	14.05	-14.57	8.54	22.57	2.64	3.08
Te-066 Ind B	2.335	14.01	-14.34	14.08	38.75	2.75	3.21
Te-070	8.23	11.24	-16.90	14.00	40.68	2.91	3.39

Note – To = Tombe adulte (adult tomb) – although some early juveniles may be designated at To if they were buried in this type of tomb.

Note – Te = Tombe enfant (child/infant tomb) located in a pyramid wall

The first determination of preservation is the percent collagen yield that is calculated after the completion of collagen extraction. This calculation determines how much collagen was present in the bone sample. The equation used to find this value is:

$$\% \text{ Collagen Yield} = \frac{Collagen\ weight\ (g)}{Sample\ dry\ weight\ (g)} \times 100 \quad (4)$$

For fresh bone, collagen is expected to take up 20% -25% of the weight of the dry bone (Schoeninger et al. 1989). With archaeological samples, however, there is some expected collagen loss and as a result many scholars have attempted to develop a minimum percentage value that should be reached for the data to be considered valid. Some scholars have advocated for a minimum of 5-6% (Schoeninger and DeNiro 1982; Tuross et al. 1988). While White and Schwarcz (1989) and White et al. (1993) suggest that only 1% collage is needed to provide reliable results, the present study used 2% (suggested by DeNiro and Weiner 1988) as the required value. There were 34 samples that fell under 2%, which can be seen in Table 7.

The next two measures of preservation are based on data provided after sample analysis. The first concerns the concentrations of carbon (wt% C) and nitrogen (wt% N) that are illustrative of the sample's quality. Ambrose (1990) found mammalian bone should have between 15% to 47% carbon, and between 5% to 17% nitrogen. The wt% C and wt% N values are reported in Table 7. Those values that fall outside of the limits are not considered for analysis. The wt% for carbon and wt% nitrogen compared to their corresponding δ values can be seen in Figures 3 and 4. There are 20 samples that have wt% nitrogen values that fall outside of the expected range, and 18 that show wt% carbon outside the accepted range. There are five samples (Table 8), however, that were included in the final analysis, due to their atomic C:N ratio values.

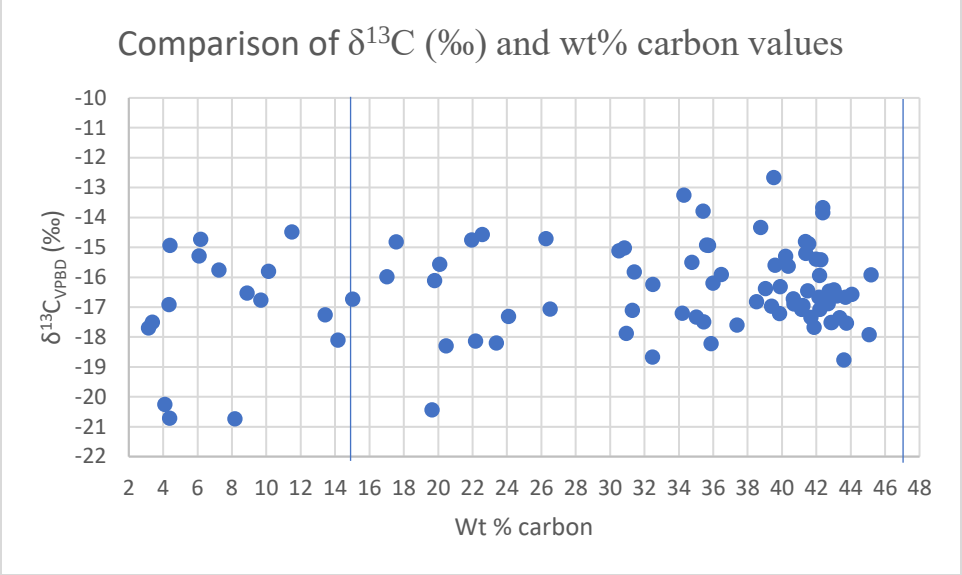

Figure 3 Graph plotting $\delta^{13}C$ (‰) values against wt% carbon. The samples located between the blue lines (15% and 47%) are considered to be preserved.

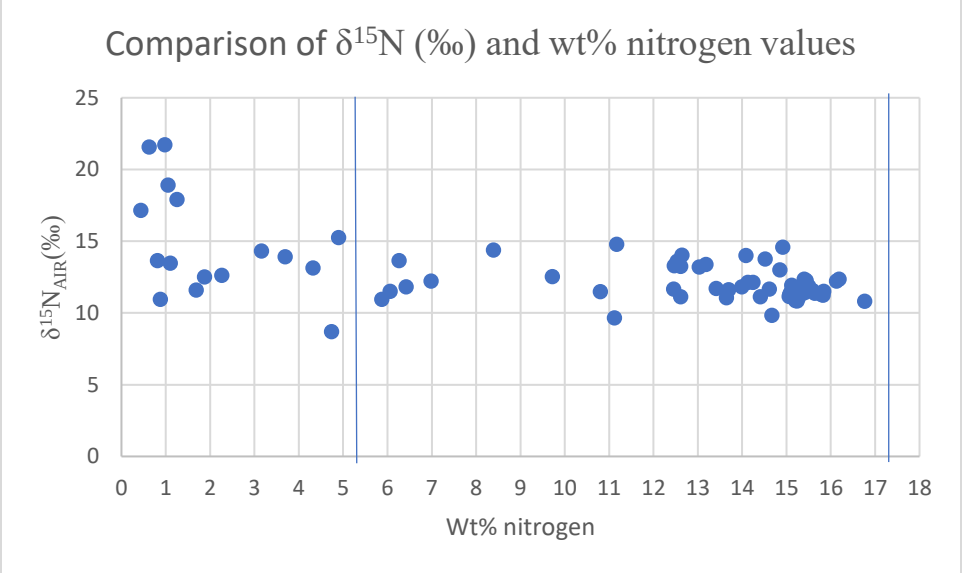

Figure 4 Graph plotting $\delta^{15}N$ (‰) values against wt % nitrogen. Those samples between the two lines (5% to 17%) are considered to be preserved.

Table 8 Meroitic Samples with either wt C% or wt N% values outside accepted ranges, but included for analysis due to their atomic C:N ratio.

Original Sample ID	Adult/ Juvenile	Age	%Collagen	wt % N	wt% C	Atomic ratio C:N
To-024	Adult	40 years	2.14	3.69	11.49	3.63
To-026C-2	Adult	35 years	1.6	4.32	13.42	3.62
To-031 Ind B	Juvenile	2 years	2.66	4.74	14.17	3.49
Te-002 Ind 1A	Juvenile	1 month	0.35	1.68	4.36	3.03
Te-003	Juvenile	40 weeks	.56	2.21	6.08	3.21

The last measure of preservation is the atomic C:N ratio, determined by this equation:

$$\text{atomic C: N} = \frac{14}{12} \times (weight\ \%\ C{:}N) \qquad (5)$$

This calculation was necessary as current mass spectrometers give C:N ratios that are lighter than previously described by DeNiro (1985), by a factor of $\frac{14}{12}$ (Katzenberg 2008). For modern bone collagen, the expected atomic C:N ratio is approximately 3.2. For archaeological bone DeNiro (1985), however, suggests a ratio that falls between 2.9 and 3.6 is an indication of good preservation. Figure 5 shows the atomic C:N ratio graphed against the percent collagen yield. Those samples falling outside the range of 2.9 to 3.6 were not considered for further analysis. Table 9 presents the average values for each preservation characteristic for both the entire samples (before samples were removed), and the sample used for final analysis.

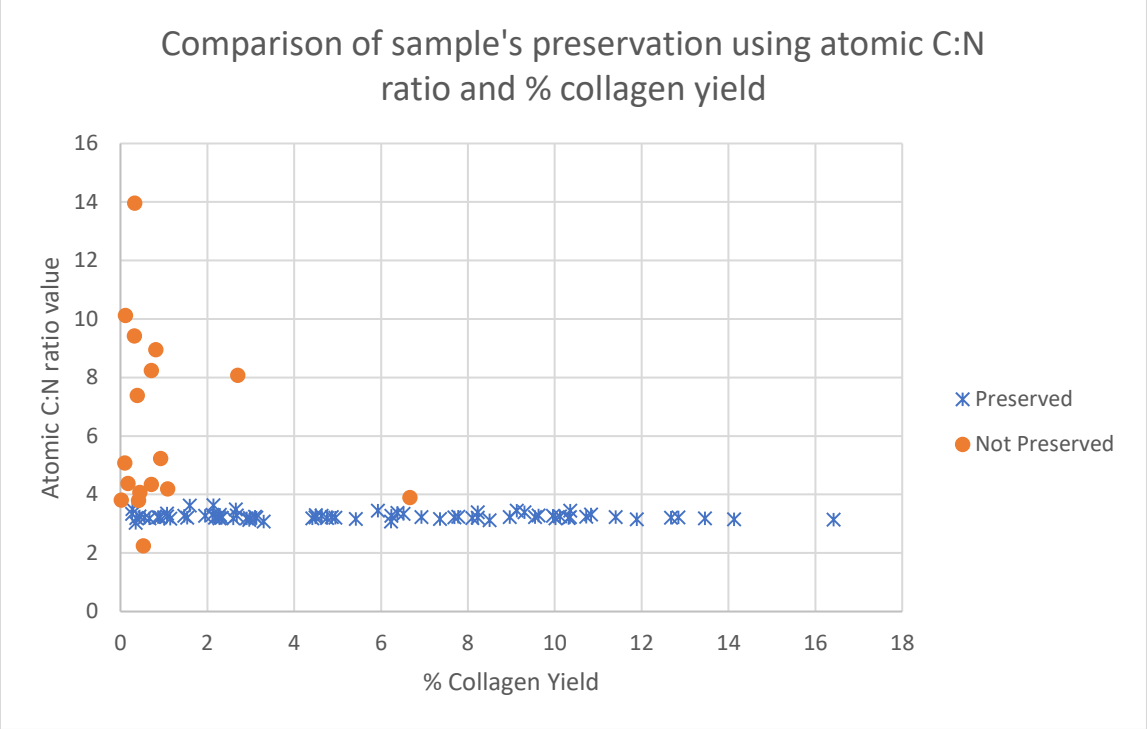

Figure 5 Graph plotting atomic C:N ratio against the percent collagen yield. The circles represent samples that are not preserved, and the stars represent those that are preserved based on atomic C:N ratio. With the exception of two samples, all samples with unacceptable C:N ratios have less than 2% collagen yield.

Table 9 Average Values for Preservation

Meroitic Samples	Average Collagen Yield	Average Atomic C:N ratio	Average Wt.% N	Average Wt.% C
All Samples (N= 100)	4.70	3.83	10.83	30.87
Preserved Samples (N=72)	5.63	3.25	12.39	34.48

Interpretation of Weaning Patterns

Stable nitrogen and carbon isotopic analysis was conducted on a total 100 samples, and after determining preservation and taking the mean values of duplicate samples, there were 31 non-adults and 41 adults used for the final analysis and interpretation of weaning patterns. While the adult isotope values will only be used as a comparison baseline, the focus of the analysis is on the non-adults to answer questions pertaining to weaning. The mean and standard deviation for stable nitrogen and carbon isotopes of the adult female samples are 11.52‰ ± 1.23 for $\delta^{15}N$ and –16.69‰ ± 1.11 for $\delta^{13}C$, and for the adult males the values are 12.18‰ ± 0.95 for $\delta^{15}N$ and –15.58‰ ± 1.36 for $\delta^{13}C$. The data for the non-adult samples are shown in Table 10, and are organized by youngest, 38 gestational weeks, to the oldest, 16 years old.

Table 10 The $\delta^{15}N$ and $\delta^{13}C$ values for non-adults by age at death and age category

Time Period	Age	Age Category	$\delta^{13}C$ (‰) (Permil vs VPDB)	$\delta^{15}N$ (‰) (Permil vs AIR)
Te-066 Ind A	38 weeks	Infant	-14.57	14.05
Te-066 Ind B	40 weeks	Infant	-14.34	14.01
Te-026	40 weeks	Infant	-15.92	11.30
Te-011 Ind A	40 weeks	Infant	-18.22	11.26
Te-013 (a)	40 weeks	Infant	-15.57	13.76
Te-014a	40 weeks	Infant	-16.53	14.32
Te-003	40 weeks	Infant	-15.29	13.33
Te-001 Ind A	40 weeks	Infant	-17.87	14.79
Te-001 Ind B	40 weeks	Infant	-18.13	14.39
Te-015 (2a)	1 month	Infant	-15.02	12.05
Te-017a	1 month	Infant	-14.88	13.76
Te-017b	1 month	Infant	-15.91	13.38
Te-002 Ind 1A	1 month	Infant	-20.71	11.58
Te-002 Ind 3	1 month	Infant	-16.19	11.70
Te-004 Ind 1A	1 month	Infant	-17.06	14.77
Te-057	1-3 months	Infant	-15.42	11.34
Te-004 Ind 2B	3 months	Infant	-16.74	12.47
To-031 Ind B	2 years	Young child	-18.11	8.70
Te-070	2 years	Young child	-16.90	11.24
Te-007/008 Ind 1A	2 years	Young child	-15.12	13.49
To-038 Ind 1	2-3 years	Young child	-15.51	13.24
Te-063	4 years	Young child	-18.67	10.29
Te-019	7 years	Older child	-16.83	12.05
Te-033	8 years	Older child	-17.22	10.62
To-018 Ind 4	8 years	Older child	-18.76	11.23
To-040 Ind 3	9-11 years	Older child	-16.45	10.86
Te-004 Ind 3	9-12 years	Older child	-17.61	11.13
To-031 Ind C	11-17 years	Older child	-17.11	9.66
To-054 Ind 1	12 years	Older child	-16.38	11.82
Te-005	13-15 years	Older child	-17.49	8.90
Te-034	16 years	Adolescent	-16.32	11.50

*Median ages used for age category placement

The $\delta^{13}C$ and $\delta^{15}N$ values of all of the preserved non-adult samples are presented in Figure 6. The average of the adult male and female $\delta^{15}N$ values are presented as solid and

dashed lines to show the difference from the non-adults. There were three adults that could not be included in the averages as it was not possible to estimate their biological sex. At this point all of the non-adults are not divided by age category, but there is a notable group of non-adults with higher nitrogen values than most of the population, which may point towards those individuals being exclusively breastfed. The next question would be if there were any patterns found when the ages of the non-adults were considered (Figure 7).

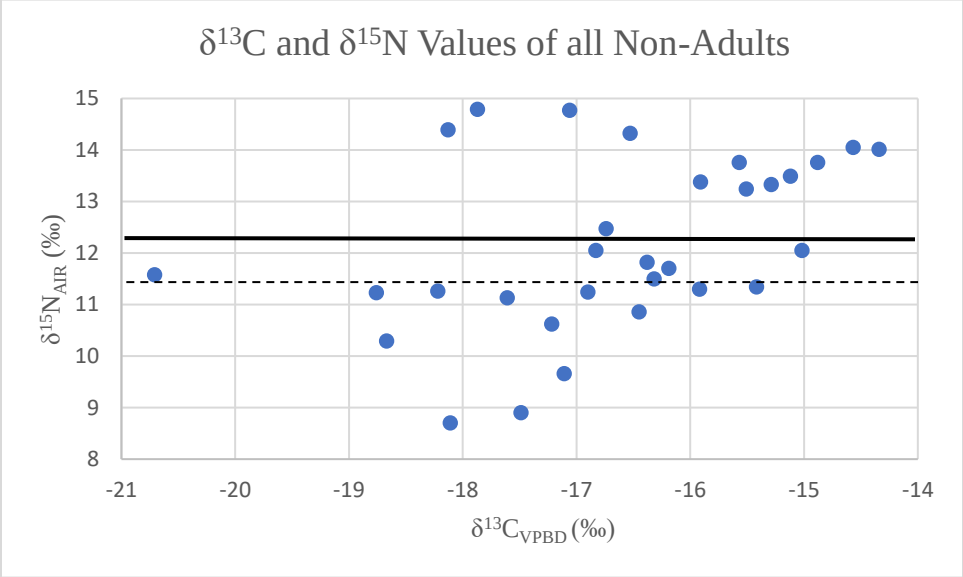

Figure 6 Graph showing the $\delta^{15}N$ and $\delta^{13}C$ values of all non-adults. The solid line represents the adult female $\delta^{15}N$ average value (11.52‰) and the dashed line represents the adult male $\delta^{15}N$ average value (12.18‰).

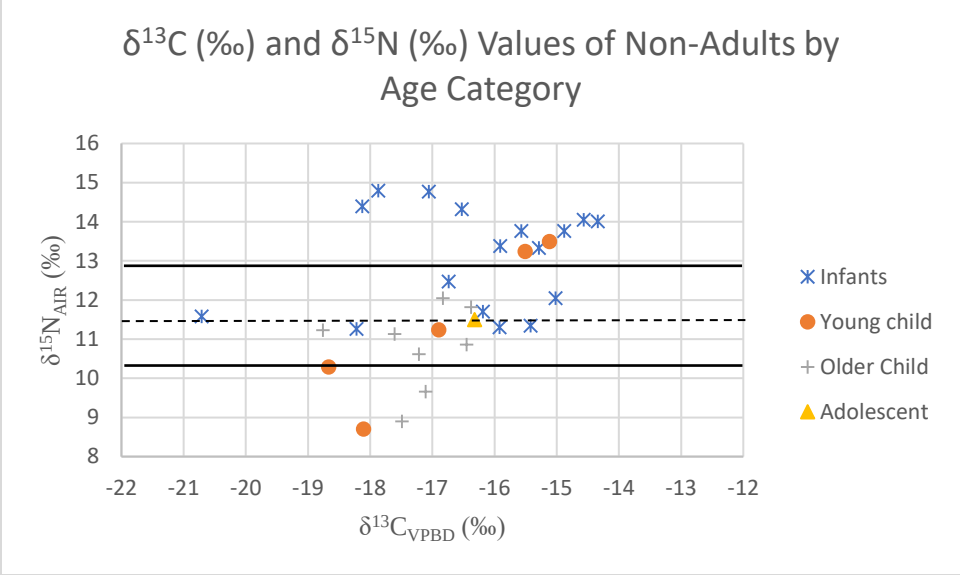

Figure 7 Graph plotting $\delta^{15}N$ against $\delta^{13}C$ values for non-adults separated into age categories. The dashed line represents the adult female mean $\delta^{15}N$ value (11.52‰) while the solid lines represent one stand deviation above and below the mean (± 1.11‰).

Non-adult individuals were spilt into four age groups discussed in the methodology chapter. These categories included infant (0-1 year), young children (1-5 year), older children (6-15 year), and adolescents (15-17 year). The age estimation ranges widen as the children get older, which may make it difficult to make precise predictions of patterns. There was one non-adult (To-031 Ind C) whose age estimation, 11 to 15 years, places them between the older child and adolescent range. Based on the median age the sample was placed in the older child group. Additionally, there was not an even number of individuals in each category, because of a limited sample size.

In general, the $\delta^{15}N$ values decrease as the non-adults increase in age. The older non-adults show the least variation in their $\delta^{15}N$ values. In comparison, $\delta^{15}N$ values of the individuals in the infant category show a greater variation, although most of the $\delta^{15}N$ values are elevated above the female mean (N= 10). Another general observation is that as the non-adults

increase in age, their $\delta^{13}C$ values tend to get more depleted, and are closer to adult female $\delta^{13}C$ values compared to the adult males. The next step is to breakdown the ages even further to try and identify any other patterns that may be missed by placing the individuals in age categories that are too broad (Figure 8).

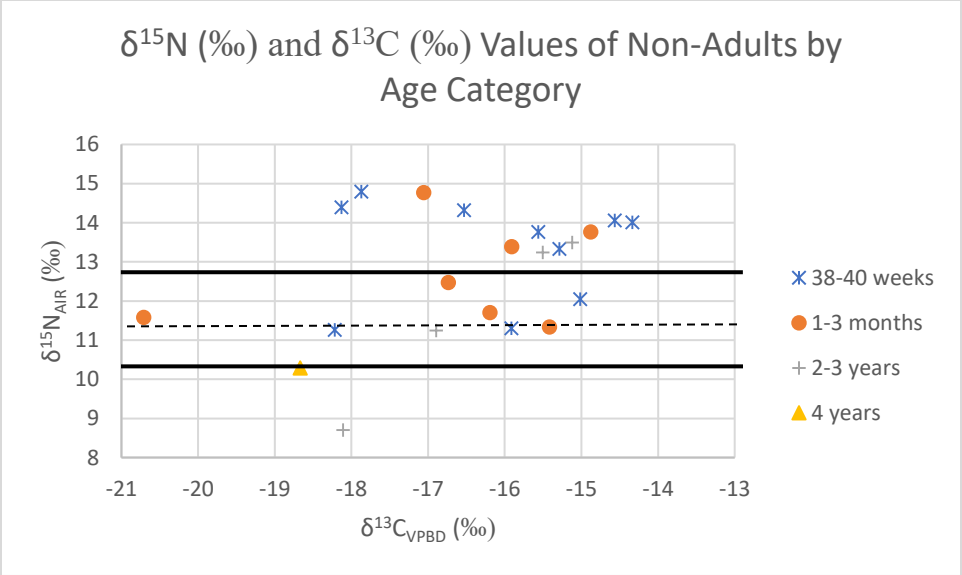

Figure 8 Graph plotting $\delta^{15}N$ and against $\delta^{13}C$ values for younger non-adults who are at an age that is typically associated with weaning. The dashed line represents the adult female mean $\delta^{15}N$ value (11.52‰) while the solid lines represent one standard deviation above and below the mean (± 1.11‰).

The age categories used in Figure 8 are a reflection of the number of individuals at certain developmental stages. For example, as shown in Table 10, there are several individuals aged to 40 gestational weeks and only one individual at 38 gestational weeks. Next, there are several individuals aged to 1-month-old, and then two individuals aged at 1 to 3 months and 3 months old, respectively. Following this, there are two 2-year-old individuals, one 2 to 3-year-

old, one 3-year-old, and one 4-year-old. There does not appear to be a pattern in the isotopic data in the non-adults who are estimated to be in the two to four-year-old range.

A difference in isotopic patterns can be detected between the 38-40 gestational week-old individuals and the 1-3 months old non-adults. Most of the individuals in the 38-40-week-old age category have higher $\delta^{15}N$ values compared to those in the 1-3 months old category. Another notable difference between the two groups is that the 1-3 months old show greater variation in their $\delta^{15}N$ values than the individuals in the 38-40- week-old age category. While there are two individuals in the 38-40 gestational week age category whose $\delta^{15}N$ values are approximately 11‰, most individuals are between 13‰ and 15‰. When considering the $\delta^{13}C$ values of these two age categories, there does not appear to be able any discernable patterns or differences. The $\delta^{13}C$ values for the 1-3-month-old non-adults cluster somewhat between -17‰ and -15‰. As noted previously, the $\delta^{13}C$ values have less variation the closer they get to adolescence. The next graphs (Figure 9-10) address how age correlates specifically to $\delta^{15}N$ values.

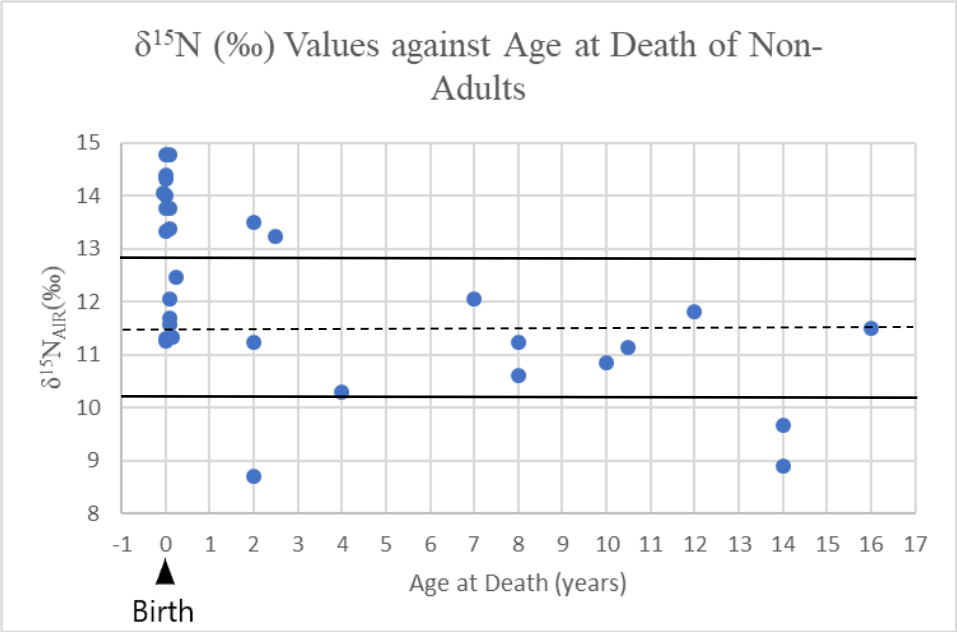

Figure 9. Graph showing the $\delta^{15}N$ values of non-adult individuals plotted against age at death. The dashed line represents the adult female mean $\delta^{15}N$ value (11.52‰) while the solid lines represent one stand deviation above and below the mean (± 1.11‰).

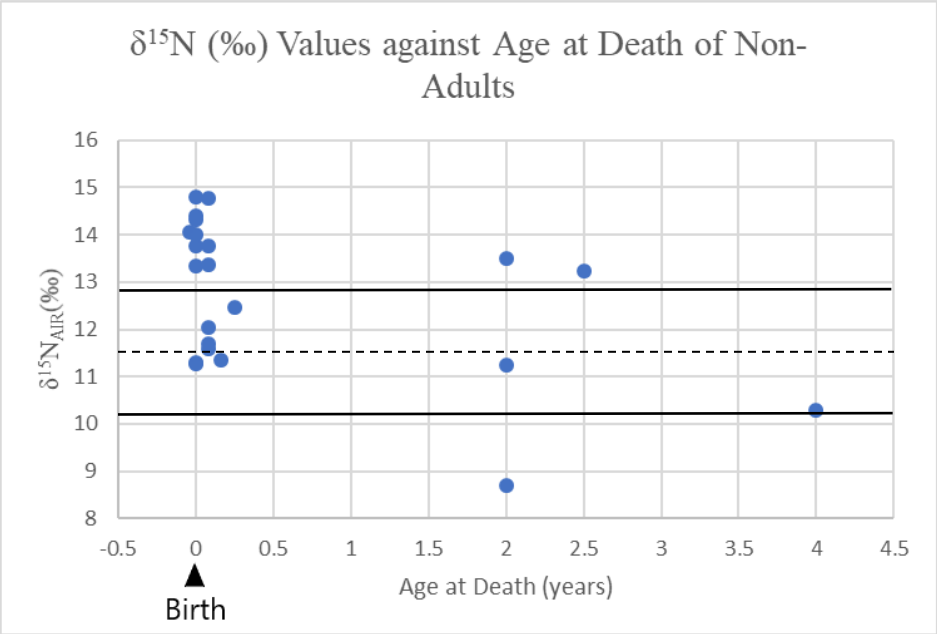

Figure 10 Graph showing the $\delta^{15}N$ values of younger non-adult individuals (38 weeks to 4 years) plotted against age at death. The dashed line represents the adult female mean $\delta^{15}N$ value (11.52‰) while the solid lines represent one stand deviation above and below the mean (± 1.11 ‰).

In considering Figures 9 and 10, any individual with a $\delta^{15}N$ value higher than one standard deviation above the mean would be considered to still be exclusively breastfeeding or beginning the weaning process. No individual past the age of 2.5 is above this line. The individuals with the highest $\delta^{15}N$ values, and farthest from the adult female average, are the individuals in the 38-40 weeks and 1-month old age categories. The individual aged to 3 months old has a $\delta^{15}N$ value that is within one standard deviation of the adult female mean $\delta^{15}N$ value, and would suggest that this individual has potentially stopped or had not been breastfed at all. There is also a group of infants that have $\delta^{15}N$ values that fall close to the average adult female $\delta^{15}N$ value suggesting that these individuals never started breastfeeding. There are no individuals past the age of 2.5 with $\delta^{15}N$ values that are one standard deviation or

more above the adult female average $\delta^{15}N$ value, indicating that by the age of 2.5 individuals may have been fully weaned.

Limited statistical analyses were conducted using a Mann Whitney U test, which is a nonparametric test that can be used with smaller samples sizes and does not assume a normally distributed sample (Table 11). These tests show that adult males and females have significantly different nitrogen values. The female adults were not significantly different from all the non-adults, but was significantly different from non-adults aged under 5 years. Lastly non-adults ages under 3 years and those aged over 3 years presented significantly different nitrogen values.

Table 11. Results of Mann Whitney U Tests for Nitrogen values

Demographic Groups	Z-score	P-value	Significant
Adult Male and Adult Females	2.20836	.0271	Yes
Female Adults and Non-adults	1.51138	.13104	No
Female Adults and Non-adults <5	2.55209	.01078	Yes
Non adults < 3 and Non adults > 3	3.2961	.00096	Yes

One consideration for weaning patterns is the type of foods that the non-adults were being weaned with, and how consistent this is across the age categories. To answer these questions the percentage of C_4 plants in the diet was calculated using the formula developed by Schwarcz et al. (1985):

$$PC4 = \frac{\delta c - \delta 3 + \Delta\, dc}{\delta 4 - \delta 3} \times 100 \qquad (6)$$

In this equation, δc is the $\delta^{13}C$ value of the sample. The average values for C_4 and C_3 plants are, represented by δ3 and δ4, respectively. The amount of fractionation, – 5‰, is symbolized by

Δdc (White and Schwarcz 1994). When choosing the values for δ3 and δ4 the environment and diet of the sample population needs to be considered to be able to calculate accurate results. As mentioned previously, a variety of factors, outside of diet, can impact isotope values. White and Schwarcz (1994) examined mummy samples from the Meroitic and Christian periods of Nubia, and as such the same values used for C_3 plants (–26.5‰), and for C_4 plants (–11.5‰) are applied to the sample in this research. The equation, with the values, would be:

$$PC4 = \frac{\delta c-(-26.5)+(-5)}{(-11.5)-(-26.5)} \times 100 \quad (7)$$

The values for $\%C_4$ for the non-adults of the population can be seen in Table 12. They are ordered from youngest to oldest. The table also has the median ages for all the non-adults, which will be used when these values are graphed due to the inability to graph age ranges.

Table 12 Percent C_4 for Meroitic Non-adult individuals

Time Period	Age	Median Age for Graphing	%C_4
Te-066 Ind A	38 weeks	38 weeks	46.20%
Te-066 Ind B	40 weeks	40 weeks	47.73%
Te-026	40 weeks	40 weeks	37.20%
Te-011 Ind A	40 weeks	40 weeks	21.87%
Te-013 (a)	40 weeks	40 weeks	39.53%
Te-014a	40 weeks	40 weeks	33.13%
Te-003	40 weeks	40 weeks	41.40%
Te-001 Ind A	40 weeks	40 weeks	24.20%
Te-001 Ind B	40 weeks	40 weeks	22.47%
Te-015 (2a)	1 month	1 month	43.20%
Te-017a	1 month	1 month	44.13%
Te-017b	1 month	1 month	37.27%
Te-002 Ind 1A	1 month	1 month	5.27%
Te-002 Ind 3	1 month	1 month	35.4%
Te-004 Ind 1A	1 month	1 month	29.60%
Te-057	1-3 months		40.53%

Time Period	Age	Median Age for Graphing	%C_4
		2 months	
Te-004 Ind 2B	3 months	3 months	31.73%
To-031 Ind B	2 years	2 years	22.60%
Te-070	2 years	2 years	30.67%
Te-007/008 Ind 1A	2-3 years	2.5 years	42.53%
To-038 Ind 1	3 years	3 years	39.93%
Te-063	4 years	4 years	18.87%
Te-019	7 years	7 years	31.13%
Te-033	8 years	8 years	28.53%
To-018 Ind 4	8 years	8 years	18.27%
To-040 Ind 3	9-11 years	10 years	33.67%
Te-004 Ind 3	9-12 years	11 years	25.93%
To-031 Ind C	11-17 years	14 years	29.27%
To-054 Ind 1	12 years	12 years	31.13%
Te-005	13-15 years	14 years	26.73%
Te-034	16 years	16 years	34.53%

A notable pattern is that most the individuals who had C_4 percent values in the 40% range were the younger individuals, while only one older individual, Te-007/008 Ind 1A, who is aged between 2-3 years of age, showed a high %C_4 value (42.5%). Additionally, the 1-month old age category had the largest range of %C_4 values with the lowest at 5.27% and highest at 47.73%. Figures 11 and 12 shows %C_4 values graphed against age at death.

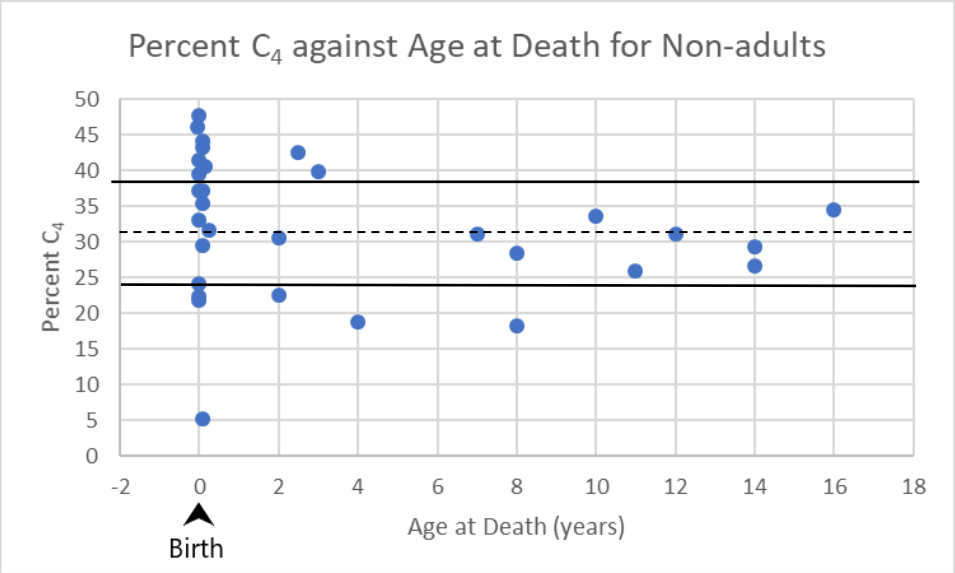

Figure 11 Graph showing percent C_4 of non-adult individuals plotted against age at death. The dashed line represents the adult female mean %C_4 value (32.07%) while the solid lines represent one standard deviation above and below the mean (±7.37%).

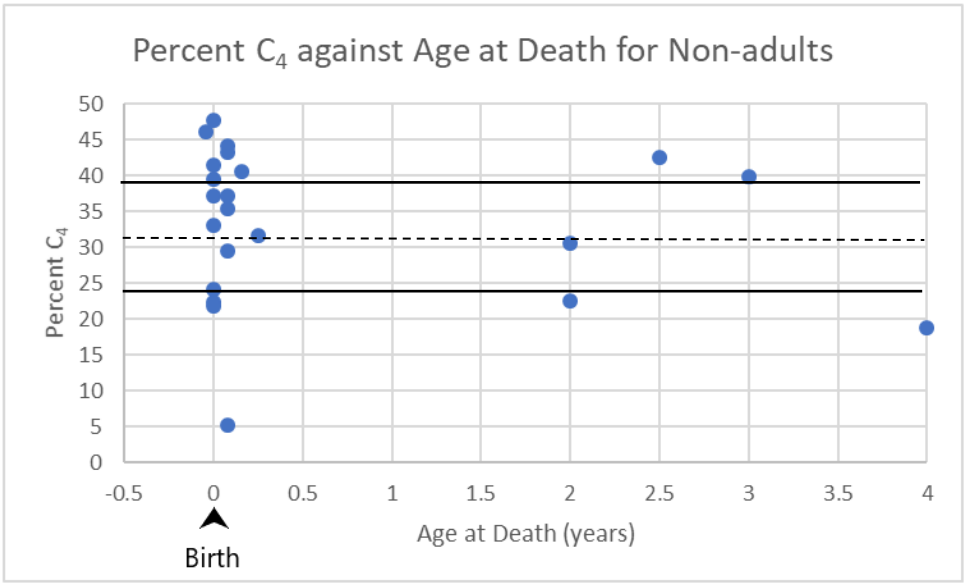

Figure 12 Graph showing percent C_4 of the younger non-adult individuals (38 gestational weeks to 4 years of age) plotted against age at death. The dashed line represents the adult female mean percent C_4 value (32.07%) while the solid lines represent one stand deviation above and below the mean (±7.37‰).

The graphs show that there is great variation in the amount of C_4 foods consumed by the youngest individuals. The highest values may also reflect the carbon trophic effect present in breastfeeding individuals or a combination of both. The older individuals show a consumption of C_4 derived foods that is closer to that of the adult females. This is particularly evident in Figure 11 where the plot points converge on the line as the individuals go up in age. One important consideration, however, is that the sample sizes are not consistent. It is possible that when more samples are added to the older age groups their values will also show this level of variation. The majority of the individuals appear to have had a diet with approximately 20% and 40% C_4 food sources.

While this research focuses on the non-adults, the adults are used for comparison. Table 13 shows the average, minimum, and maximum percent C_4 values for the male and female adult groups. Differences between these groups could indicate a gender-based diet. Based on these values, it can be noted that there are both male and female adults who have higher percent C_4 values, in the 50% range, compared to the non-adults.

Table 13 Adult Male and Female %C_4 values

Sample Group	**Average %C_4**	**Minimum %C_4**	**Maximum %C_4**	**Standard Deviation**
Adult Males	40.84%	26.47%	58.93%	10.15
Adult Females	32.09%	23.87%	51.47%	7.37

Figures 13 and 14 show a potential relationship between the $\delta^{15}N$ value and the percent C_4. Figure 13 shows $\delta^{15}N$ values plotted against percent C_4 values for non-adults by age categories. This graph demonstrates that while there is a wide range in percent C_4 values for

the youngest individuals, they also show the highest $\delta^{15}N$ values. This is important because if the individuals who were most likely breastfeeding, shown by high $\delta^{15}N$ values, also have higher $\delta^{13}C$ values than those who appear to have ceased breastfeeding, it could indicate particular weaning foods.

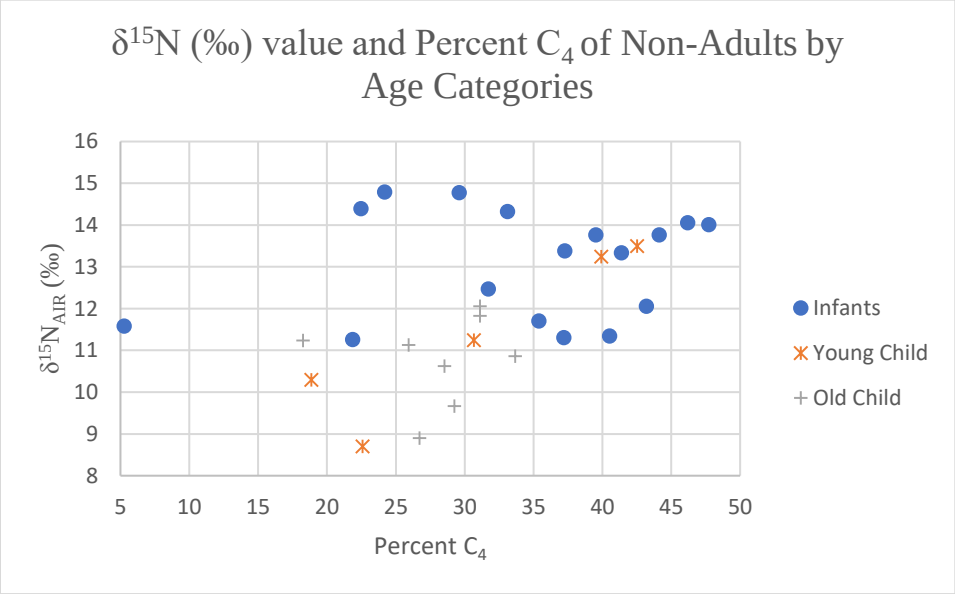

Figure 13 Graph showing $\delta^{15}N$ values plotted against percent C_4 values of all non-adult individuals separated by age categories.

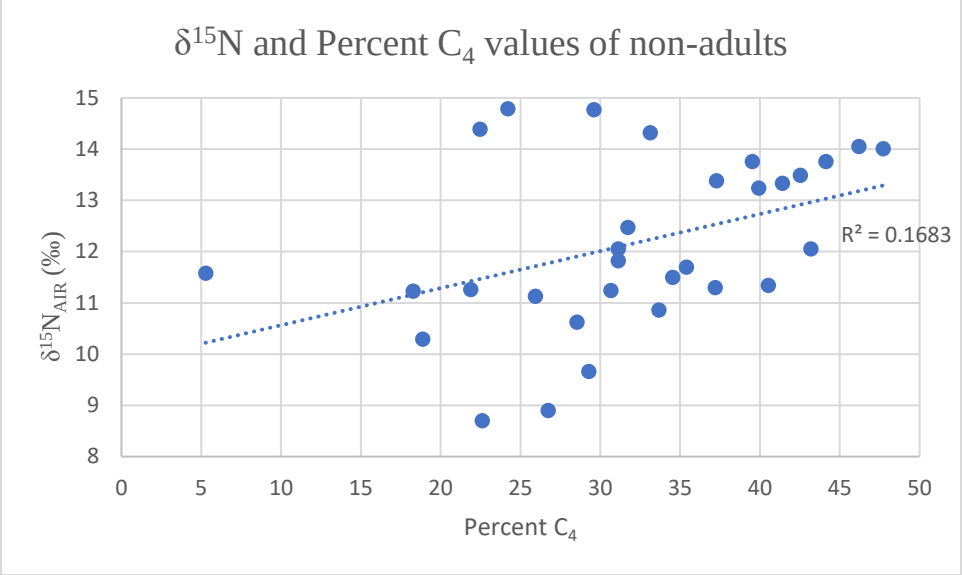

Figure 14 Graph showing $\delta^{15}N$ values plotted against percent C_4 values of all non-adult individuals. The dotted line represents the trend line.

Figure 14 shows $\delta^{15}N$ values plotted against percent C_4 values for all non-adults, with a trend line and R-value ($p < .05$), which shows how close the data is to the regression line. The trend line and R^2-value (0.1683) show a positive correlation between these values. The higher the $\delta^{15}N$ value, the more C_4 seen in the individual's diet. While it would be impossible to use this trend line in a predictive function, it demonstrates the potential relationship in this population of breastfeeding practices coupled with the consumption of C_4 based food sources.

As with nitrogen, a Mann Whitney U test was used for statistical analysis (Table 14) of stable carbon isotope values. These tests show that adult males and females have significantly different carbon values. The female adults were not significantly different from all the non-adults or the non-adults aged under 5 years. This group did, however have significantly

different nitrogen values. Lastly non-adults ages under 3 years and those aged over 3 years presented significantly different carbon values.

Table 14 Results of the Mann Whitney U test for Carbon Values

Demographic Groups	Z-score	P-value	Significant
Adult Male and Adult Females	2.64138	.0083	Yes
Female Adults and Non-adults	0.46865	.63836	No
Female Adults and Non-adults <5	0.81976	.41222	No
Non adults < 3 and Non adults > 3	2.0495	.04036	Yes

CHAPTER FIVE: DISCUSSION

This chapter presents a discussion of the previously presented data to contextualize it within culture, biology, and isotopic and bioarcheological theory with the ultimate aim of understanding the weaning patterns of this elite Meroitic population. The discussion will focus upon the previously presented research questions. What age were infants being weaned? What potential foods were being used as weaning foods to supplement breastfeeding? And what does this information tell us about this population? The interpretation of the results will also consider socioeconomic status, gender norms, and potential illness.

Although individual values are used to interpret weaning patterns, average values for $\delta^{15}N$ and $\delta^{13}C$ values and $\%C_4$ will also be presented throughout to help "reduce the noise" (Reynard and Tuross 2015: 620). In some cases, utilizing averages can help to show a more accurate representation of the population; however, a sufficient sample is needed for this. This will be taken into consideration when analyzing the data, as the samples sizes, when broken down into age categories, are small.

At what age are individuals being weaned?

As discussed in previous chapters, stable nitrogen isotope values can be used to estimate weaning patterns (Fogel et al. 1989; Katzenberg and Pfeiffer 1995; Fuller et al. 2006), and help to identify the types of food consumed. In general, stable nitrogen isotope values will increase as the consumer moves up a trophic level on the food chain. This directly impacts the tissue of breastfeeding and weaning infants because when an individual is being breastfed, they

are technically higher up the food chain than their mother. Typically, the stable nitrogen isotope values of an exclusively breastfed infant will be about 2-3‰ higher than their mother (Katzenberg and Pfeiffer 1995). As supplementary food is introduced to an infant's diet their stable nitrogen isotope values should begin to decrease (Fogel et al. 1989).

Since the stable nitrogen isotope values of breastfed non-adults are influenced by their mother's nitrogen values, it is important to compare the stable nitrogen isotope values of the non-adults to the average value of the female adults. This is especially true for the 8-B-5.A cemetery because infants were buried isolated from adults, and non-adults could not be biologically associated with specific adult females. As such, an average isotope value for adult females is used for a base line. Table 15 presents descriptive statistics of non-adults, separated by age category, and the adults of the population, which are separated by sex. The male adults were included to examine connections with adult diet in general.

Table 15 Descriptive Statistics for $\delta^{15}N$ values of adults and non-adults

Age	Sample Size	Minimum Value	Maximum Value	Average $\delta^{15}N$ (‰) Values	Standard deviation
Adult Female	15	8.62	14.02	11.52	1.23
Adult Male	24	11.06	14.59	12.18	.95
All Non-adults	31	8.7	14.79	12.16	1.67
38-40 Weeks	9	11.30	14.79	13.47	1.31
1-3 Months	8	11.34	14.77	12.63	1.22
2-3 Years	4	8.7	13.49	11.67	2.22
4 Year	1	N/A	N/A	N/A	N/A
7-15	8	8.9	11.82	10.78	1.06
16	1	N/A	N/A	N/A	N/A

The average $\delta^{15}N$ value for all the non-adults was 12.16 ‰, which slightly higher than the average for adult females of 11.52‰. The average for all non-adults, however, represents a very wide age range and developmental stages, and as such it is more appropriate to consider the data of non-adult individuals by age categories. The youngest individuals, 38-40 gestational weeks-old, have an average $\delta^{15}N$ value of 13.47‰, which is approximately 2‰ higher than the mean adult female $\delta^{15}N$ value. It is also important to note that the range of $\delta^{15}N$ values in this group indicate some individuals were 3‰ higher than the average adult females. This aligns with literature in that an infant who is being solely breastfed would have $\delta^{15}N$ values about 2-3‰ higher than their mothers. Others, however, had almost the exact same values as the adult females. One possible interpretation is that these died before breastfeeding had started.

It also important, given the early age, to consider the implication of bone turnover rate, which was discussed previously. Limited information is known about non-adult bone growth rate. As a result, the values presented may be from when the individual was developing in utero (Beaumont et al. 2015). In has been shown that mothers who experience nutritional stress during their pregnancy can have raised nitrogen levels (Beaumont et al. 2015). Some of the individuals who appear to be breastfeeding could really be showing an ill or undernourished mother. In addition, the statistical tests showed that individuals aged less than 5 years had significantly different nitrogen isotope values compared to the adult females, but not significantly different carbon isotope values. If they were breastfeeding non-adults of this age would have different nitrogen isotope values, but the carbon isotope values would be similar, which is shown in the statistics. If there was a larger sample size, carbon isotope values of infants with higher nitrogen isotope values could be compared to those of the adult females to test if weaning had begun or if they were still potentially reflecting their mother's values.

The next age category encompasses 1 to 3-month-old individuals; however, this age category is mostly composed of 1-month-old individuals. Potential error in aging should be acknowledged. The average $\delta^{15}N$ value for this group was 12.63‰, which is approximately 1‰ higher than the mean adult female $\delta^{15}N$ value. The average $\delta^{15}N$ value for this group indicates that the diet of these individuals was already changing, and most likely supplemental foods were being introduced, signaling the beginning of weaning. This, however, is a very early age for an individual to start the weaning process. This might occur because the mother died and was unable to continue feeding or as mentioned above it is reflective of value from gestation.

Figure 15 shows that approximately half of the individuals in the 1 to 3-month category are showing nitrogen isotope values that are 2-3‰ higher than the adult female baseline (the dashed line) and some have values that are similar. This could indicate that some infants were still exclusively breastfeeding at this age and others began to wean. The individuals with $\delta^{15}N$ values closer to or at the adult average $\delta^{15}N$ value may not have breastfed at all, or were fed supplemental milk from animals, perhaps as an illness food. At this age, individuals would have been too young to eat solid foods. Another important consideration is maternal mortality, where the death of a mother during childbirth would mean that an infant may not have been breastfed.

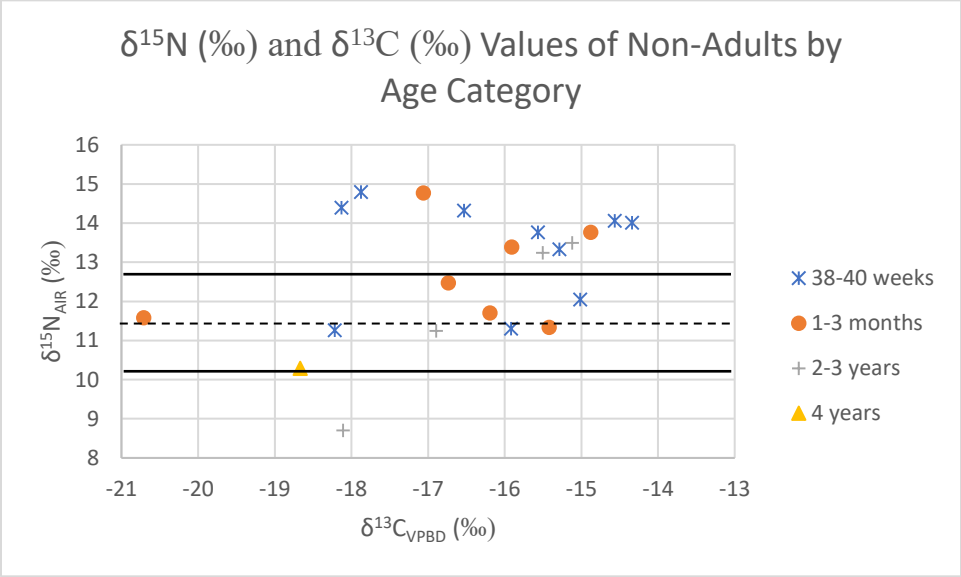

Figure 15 Non adult age categories graphed by $\delta^{15}N$ and $\delta^{13}C$ isotope values. The dashed line represents the average adult female $\delta^{15}N$ value (11.52‰), while the solid lines represent one standard deviation above and below the mean (± 1.11‰).

At around 2 to 3 years of age, the average $\delta^{15}N$ value decreases to 11.67‰, which is similar to the average female $\delta^{15}N$ value of 11.52‰. This may suggest that the weaning process stopped 2 to 3 years of age. Additionally, the Mann-Whitney U test showed that the

nitrogen and carbon values of non-adults ages below and above 3 years were significantly different. This shows that after 3 years of age there was a trophic level change and that individuals were consuming different types of food then their mothers. If they were still breastfeeding the carbon values would be different. It should be noted, however, that the sample size is small with only four individuals in this age category.

Eerkens et al. (2018), who reported on weaning timing in the same population, found an average weaning age of 2.7 ± .95. Their data was based on incremental analysis of adult dental samples. Although the Eerkens et al. (2018) study is based on 10 individuals, the results from this study support this finding, that non-adults were typically weaned by 3 years of age. This age of weaning is also similar to those reported in other studies of the same time period and wider geographical area (e.g. Nehlich et al. 2011; Keenleyside et al. 2009; Dupras and Tocheri 2007). Given the smaller samples sizes a wider range was given for potential weaning age, a general estimation from the sample is between 2 and 4 years of age.

What foods were used during weaning?

This next section examines, based on interpretation of $\delta^{13}C$ values, what potential food types may have been used to supplement the weaning process. Table 16 shows the average $\delta^{13}C$ values for adults, separated by sex, and non-adults, separated by age categories.

Table 16 Descriptive Statistics based on $\delta^{13}C$ values for non-adults and adults

Age	Sample Size	Minimum Value	Maximum Value	Average $\delta^{13}C$ (‰) Values	Standard deviation
Adult Female	15	-17.92	-13.78	-16.69	1.11
Adult Male	24	-17.34	-12.66	-15.58	1.36
All Non-adults	31	-20.71	-14.34	-16.67	1.42
38-40 Weeks	9	-18.22	-14.34	-16.14	1.50
1-3 Months	8	-20.71	-14.88	-16.70	1.87
2-3 Years	4	-18.11	-15.12	-16.41	1.37
4 Year	1	N/A	N/A	N/A	N/A
7-15 Years	8	-18.67	-16.38	-17.23	.76
16 Year	1	N/A	N/A	N/A	N/A

The average female adult $\delta^{13}C$ value is –16.69‰ and the total non-adult sample's average is almost identical at –16.67‰. The adult male's samples had the lowest average $\delta^{13}C$ value, –15.58‰. The similarity in the average $\delta^{13}C$ values between adult females and non-adults supports the idea these two groups had similar diets, most likely because they were more often in proximity to one another. This would be especially true if the infants were still being exclusively breastfed because their carbon values would be reflective of their mother's (Humphrey 2014), although a small enrichment due to trophic level is typically reported (Fuller et al. 2006). It can also be noted that there are very little differences in the average $\delta^{13}C$

values between the non-adults age categories until the older non-adult category. The individuals in the 1 to 3-month-old age category have the largest variation in $\delta^{13}C$ values. Important to note is that the 1 to 3-month-old age category has twice as many samples as the 2 to 3-year-old age category, which may have an influence on the variation.

Eerkens et al. (2018) found that Meroitic non-adults from the same cemetery had a much higher percentage of C_4 plants in their diet compared to non-adults from other regions of the same time period. They suggest that sorghum and millet-based foods, specifically, made up a substantial part of the non-adult diet in the elite Meroitic, and claimed that this diet was continued into the first decade of their life. Calculations for the percentage of C_4 plants in the diet will also be considered (Table 17). Caution must be taken however, as Thompson et al. (2008) noted, this model is not always applicable to a human diet because human diet is omnivorous and complex. Additionally, seasonal changes in diet are also observed for this area (Thompson et al. 2008).

Table 17 C_4% Values Separated by Age and Sex

Sample Group	Average % C4	Minimum% C4	Maximum % C4	Standard deviation
Adult Males	40.84%	26.47%	58.93%	10.15
Adult Females	32.09%	23.87%	51.47%	7.37
All Non-adults	32.30%	5.27%	47.73%	9.40
38-40 weeks old	35.78%	21.87%	47.73%	10.02
1-3 months	32.00%	5.27%	44.13%	11.63
2-3 years	33.93%	22.60%	42.53%	10.71
7-15 years	28.08%	18.27%	33.67%	4.53

*** Four-year-old not included due to only one value: 18.87%**

First it should be noted that the average percent C_4 value of the 38 to 40-week old individuals are similar to the average adult female values, as would be expected as the calculation relies on $\delta^{13}C$ values. This would suggest that at this age, most of the infants were not consuming supplementary foods but have $\delta^{13}C$ values that reflect the consumption of breastmilk. The next age category, 1 to 3-months-old, shows significant variation in C_4 values (5.27% to 47.73%), and differs somewhat from the adult females means. The variation in this age category may suggest, as with the $\delta^{15}N$ values, that infants of this age may have consumed other foods and were not solely getting their protein from breastmilk. The variation between individuals in this age category suggests some individuals were being weaned, perhaps with different foods, and others were still consuming solely breast milk. However, these values still may be reflective of their time in utero. It may be that the pregnant mothers were feeling sick and ate differently and this impacted the infants analyzed.

Eerkens et al. (2018) found more variation in the consumption of C_3 and C_4 foods in individuals of post-weaning ages, particularly those in the 4 to 5 age range. The authors attributed this to different children responding better to certain weaning foods. Essentially, when varying values are shown it suggest that parents with the same group had differing plans to help their child survive (Quinlan 2007). Eerkens et al. (2018) suggest that as the individuals moved out of the age ranges associated with pathological risk their diets became much more consistent with the rest of the population. This was also seen with the Egyptian samples analyzed by Dupras and Tocheri (2007). While this study's samples are not in the 4 to 5 age range, Eerkens et al. (2018) samples were of those who survived into adulthood, and therefore may not have needed supplemental foods as soon as those in this study. Another explanation to consider is that as the children aged, they spent more time together and away from their mother, and were eating the same types of foods.

One issue with the interpretation of this data, particularly for the understanding of consumption of C_3 versus C_4 foods, is that there are no food webs available for this population. The archaeological excavations of this cemetery site offered very little in faunal or floral remains, and the location of the contemporaneous habitation site has not yet been located. In this case, literature citing known floral and faunal material from other contemporaneous sites must be used to create a potential food web (Figure 16). As indicated in Chapter Two, the potential sources of C_4 foods would be millet and sorghum and milk from cattle or goats who consumed C_4 plants.

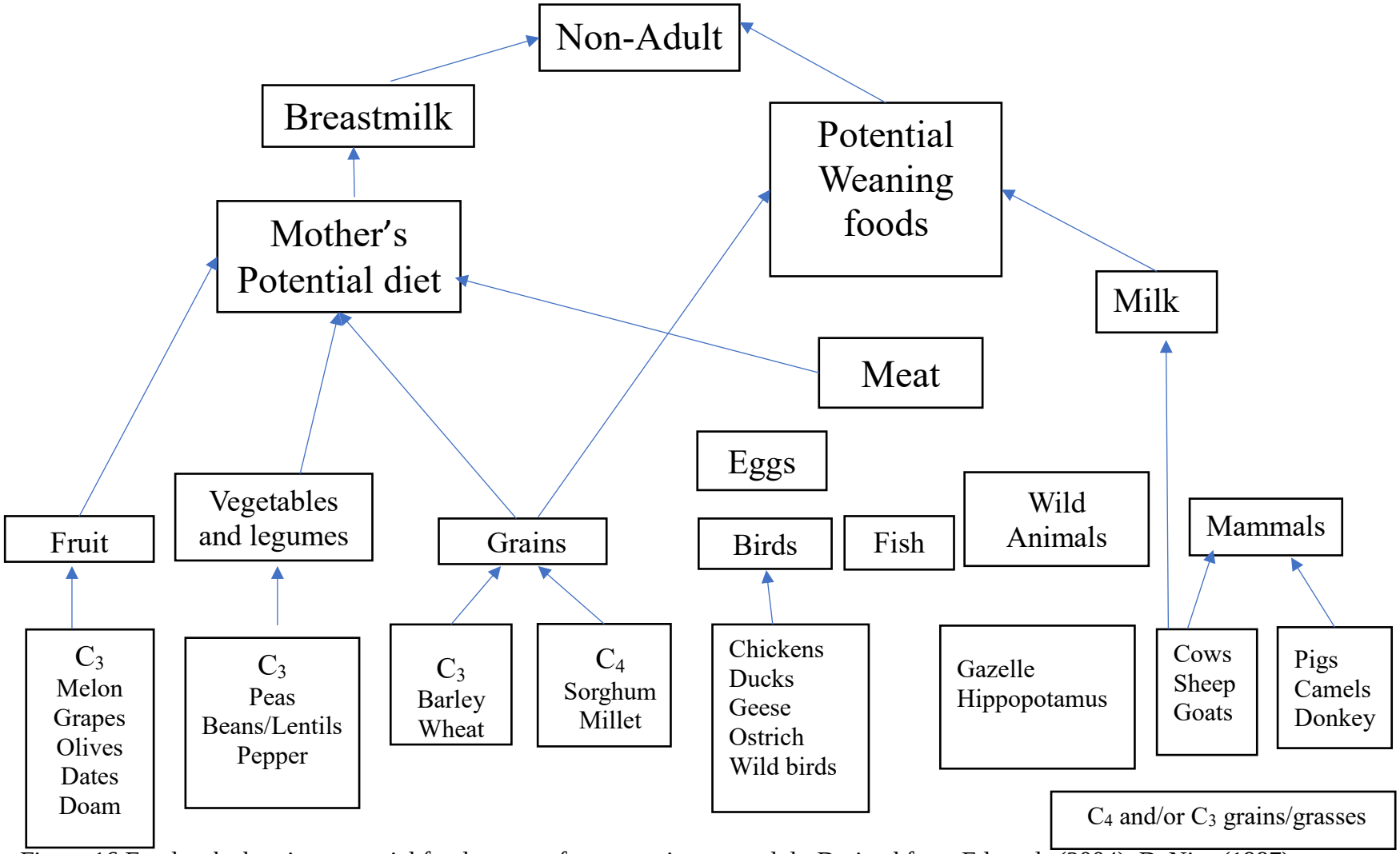

Figure 16 Food web showing potential food sources for a weaning non-adult. Derived from Edwards (2004), DeNiro (1987), Fisher (2012), Haaland (2012; 2014), Iacumin et al. (1998), and Ikram (2012).

It also important to consider the impact of season on food availability. Some individuals analyzed were born and died within one season and this may have impacted what foods they had access to. This could be a factor in the variation of carbon isotope values seen in younger individuals, and as it is not known when they died this variable cannot be controlled. Seasonality of foods have been shown to lead to deficiencies in key bodily elements, such as iron and zinc, which has been attributed to increased death of individuals under 5 years of age (Caulfield et al. 2004). Additionally, seasonality has been thought to be a factor in human health and interactions between individuals and pathogens (Nelson et al. 2002). Some seasonal diseases of ancient populations have been identified as typhus, malaria, and tuberculosis (Scheidel 2001). At present it is impossible to determine exactly how diseases manifested in different seasons on Sai Island and how this would have impacted feeding patterns.

What factors are believed to influence these decisions?

This section will outline how additional factors must be taken into consideration when interpreting the results. There are no historical records available from the site, so these are generalizations based on these data and general weaning customs with a larger consideration for contemporaneous populations. The specific factors will include socio-economic status and cultural practices regarding gender.

One consideration is that these individuals were born into the elite status of the population and their access to resources may have impacted when they began and finished weaning. Non-adults in this sector of the population could have had access to more nutritional

supplementary food, potentially wet nurses, and health care that would allow for shorter weaning periods. This pattern was observed in a historical study conducted by Katzenberg and Pfeiffer (1995), who found shorter weaning periods in the more elite members of the population. At this point generalizations can be made; however, more samples, with a larger range of ages, would be needed to give greater confidence to the present findings. In addition, at this time there are no individuals from the non-elite class to compare against to see if this pattern is related to their status.

The elite status of this population may have also impacted the food that the non-adults would have had access to because their parents most likely had access to unique trade items (Haaland 2014). Eerkens et al. (2018) also noted that there was more variation in $\delta^{13}C$ values for these Meroitic non-adults than those reported for the non-adults from the Egyptian sites of Kellis and El Hesa. Eerkens et al. (2018) postulate that this variation was due to resource availability and socioeconomic status. They also suggest the variation may have been impacted by the season in which the individual was born. Because Eerkens et al. (2018) used incremental sampling of dentition from adults to determine infant weaning patterns, they were also able to consider weaning in relation to sex.

Eerkens et al (2018) found that females may have been weaned longer, however, due to the sample size this was not definitive. Additionally, the Mann Whitney U test found a significant difference in the carbon and nitrogen values of the male and female adults. This may suggest this population differences in dietary patterns due to gender, which may impact weaning times and/or food used for weaning. Although sex cannot be estimated for this study,

sex differences may explain some of the variation found. A summary of the analysis can be seen in Table 18.

Table 18 Summary of Discussion

Age of Weaning	Types of Food	Considerations
Finished weaning around 2 to 4 years of age. Onset of weaning not clear. Some appear to begin at 1 month.	Increased dietary variation in younger children A mix of C_4 and C_3, but more C_3 C_4 foods could be linked to millet or sorghum (pap or animal milk)	**Status:** different than the other members (lower classes) of the community **Sex (Gender):** Could have impacted age of weaning

CHAPTER SIX: CONCLUSION

The goal of this research is to analyze the weaning patterns of an elite Meroitic population using stable nitrogen and carbon isotope values from bone collagen. The first question of this research was, "can the age of weaning be determined?" The hypothesis was that the children in this community would be fully weaned by 2.5 years of age, based on previous research from the same cemetery (Eerkens et al. 2018). The present research supports this hypothesis, as the isotope data suggests a weaning cessation age of between 2 to 4 years of age. While some individuals appear to have been weaned by 2.5 years, and support the previous research, there were individuals that appeared still be breastfeeding, to some degree, at later ages (Te-007/008 Ind 1A; To-038 Ind 1), although illness should also be considered as a mechanism for elevated $\delta^{15}N$ values. While the data from this study appears to closely align with that of Eerkens et al. (2018), it should be noted that this study represents a cross-sectional study, while Eerkens et al. (2018) presents individual longitudinal data from individuals who survived into adulthood, which may have impacted the weaning investment. The individuals who make up the present study died young, and these individuals may have experienced different feeding habits linked to health and the inability to thrive.

The second research question asked was, "what food was used for weaning?" The hypothesis, based on literature which described food patterns of the time (Eerkens et al. 2018), was that a variation of C_4 and C_3 plants were used for weaning. The data from this study supports this hypothesis in that younger non-adults had a high degree of variation in the percent of C_4 foods in their diets, especially the 1 to 3-month-old non-adults, with percent C_4 values ranging from 5.27% to 40.53%. The younger non-adults also exhibited more C_4 that the

older non-adults, but not more than the adults, especially the males. Factors which may have influenced diet at this time include access to food, and personal preference of the child and the beliefs of the parents at a time of high stress for the weaning infant. The hypothesis also suggested that C_3 would make up more of their diet which was supported, as no individual had a percent C_4 value above 50%.

The final research question was, "what factors influence these decisions?" The hypothesis was that socio-economic status, health, and gender (sex), would influence these decisions. It was not possible to answer this question based solely on the isotope data. While all factors could potentially influence the decision on how long to wean and what foods to introduce, only postulations can be made. Gendered difference could not be determined because sex cannot be estimated reliably from these non-adult skeletons. In order to understand the impact of socioeconomic status, a comparison with lower classes would be needed.

Limitations

The main limitations of this study are the demographics and size of the sample particularly in each age category. While there is a good range of non-adult ages there are still large gaps, which would help to estimate when weaning may have started and completely stopped. For example, having additional individuals aged at 6 months or even a year would have been valuable. While there were some individuals that were estimated to be 1 years old (To-049 Ind 2A; Te-035), these samples were not preserved. It is important to note that most of the non-adult elite individuals were buried in the pyramid walls, and over time these walls disintegrated so that only three to four courses of bricks were left standing. In many cases the

non-adult burials were exposed or had very little covering before excavation. This leads to biases in the number of burials that survived, and also issues with skeletal preservation. For example, of 35 non-adults originally analyzed, only 31 were preserved well enough to be included in the final analysis. So, although this is a limitation of the study, those individuals that were available for analysis were included.

Thus, the limitation in sample size, particularly age categories, is noted, and caution is taken to not make any sweeping generalizations. Additionally, age estimations are always with potential error and individuals could possibly be places in the wrong categories. Lastly, as previously mentioned, these individuals died in childhood and may not represent the norm. However, these data should not be dismissed as sick children may have been treated differently and this variable weaning pattern could potentially yield insight into medical care of the time.

Future Research

As discussed previously, a more extensive sample size should be used in future studies if available. In addition to having a wider range of ages, a comparison to another social class from this site may also lead to significant findings regarding social status. A significant difference in the median weaning ages between the populations would be evident of difference between socio-economic groups. Another factor to consider in future studies is a consideration of pathological conditions (although none beyond enamel hypoplasia were noted for this group).

In addition to sample consideration, the type of tissue examined with stable isotopes may be explored. For example, using skin or nails may be helpful because of the quick

turnover rate (Beaumont et al 2015). This may help account for the issues considered in discussion chapter regarding the slow turnover rate of the bone collagen. There are nail and hair samples of some of the individuals that could be analyzed and compared.

Another tissue that has been proven effective for weaning patterns is collagen from teeth (Eerkens et al. 2018; Beaumont et al. 2015). One benefit is that weaning patterns can be determined for individuals who survived into adulthood. Studies have shown the possibility of looking at segments of the tooth to discern at what age the collagen was added (Beaumont et al. 2015; Dupras and Tocheri 2007; Eerkens et al. 2018). This is because teeth form at distinct times and are not impacted by environmental stress the same way that bone would be. Teeth begin to form in utero, as well, so information about the mother's health could be obtained. As mentioned previously, a potential study could compare those who died in childhood to adults who survived through this period.

APPENDIX: RAW DATA

Table 19 Data of All Samples

Original Sample ID	Adult/ Juvenile	Sex	Age	% Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	Atomic ratio C:N
To-001 Ind 5	Adult	Male	35-50 years	8.50	11.55	-17.34	15.59	41.68	2.67	3.12
To-004 Ind 1	Adult	Male	25 years	0.37	11.66	-17.20	12.45	34.2	2.75	3.20
To-004 Ind 2	Adult	Female	young adult	2.98	12.35	-15.94	15.4	42.19	2.74	3.20
To-005 Ind 1	Adult	Male	40 years	12.85	11.50	-17.53	15.84	43.74	2.76	3.22
To-005 Ind 2	Adult	Male	45 years	13.46	12.21	-16.57	16.13	44.07	2.73	3.19
To-005 Ind 3	Adult	Male	30 years	7.36	11.87	-15.40	15.50	41.98	2.71	3.16
To-017 Ind 2	Adult	Female	45-50 years	14.13	11.15	-16.72	15.06	40.65	2.70	3.15
To-017 Ind 3	Adult	Female	20 - 29 years	11.89	12.34	-16.67	16.18	43.68	2.70	3.15
To-017 Ind 4	Adult	Male	45 years	10.32	12.26	-13.84	15.44	42.37	2.74	3.20
To-017 Ind 5	Adult	Female	old adult	7.78	11.13	-17.33	12.61	35.01	2.78	3.24
To-018 Ind 1	Adult	Male	40 years	8.23	11.42	-13.67	15.41	42.37	2.75	3.21
To-018 Ind 2	Adult	Female	20 years	16.42	10.81	-17.92	16.76	45.06	2.69	3.14
To-018 Ind 3	Adult	Female	30 years	6.93	11.94	-17.67	15.12	41.87	2.77	3.23
To-018 Ind 4	Juvenile	N/A	8 years	12.68	11.23	-18.76	15.82	43.59	2.76	3.21
To-024	Adult	Male	40 years	2.14	13.92	-14.49	3.69	11.49	3.11	3.63
To-024	Adult	Male	40 years	0.33	13.65	-16.76	0.81	9.67	11.96	13.96
To-026A	Adult	Female	35 Years	0.178	11.82	-17.31	6.42	24.09	3.76	4.38
To-026B	Adult	Male	18 years	4.87	11.35	-16.82	13.92	38.51	2.77	3.23
To-026C-2	Adult	Unknown	35 Years	1.6	13.12	-17.26	4.32	13.42	3.11	3.62
To-027	Adult	Female	25 - 30 years	0.422	14.57	-14.71	8.06	26.27	3.26	3.80
To-027 Ind 1	Adult	Female	25 - 30 years	4.76	11.28	-17.36	15.79	43.36	2.75	3.20
To-027 Ind 2	Adult	Female	28 - 34 years	2.28	11.12	-16.97	14.41	39.39	2.73	3.19
To-027 Ind 3	Adult	Female	19 years	2.91	8.62	-16.43	15.71	43.01	2.74	3.19
To-027 Ind 4	Adult	Male	35 - 44 years	1.47	11.50	-15.98	6.06	17.01	2.81	3.27

Original Sample ID	Adult/ Juvenile	Sex	Age	% Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	Atomic ratio C:N
To-027 Ind 5	Adult	Male	35 years	4.63	13.65	-14.82	6.26	17.55	2.80	3.27
To-027 Ind 6	Adult	Unknown	25 - 28 years	2.29	12.22	-16.11	6.98	19.79	2.84	3.31
To-028 Ind 1	Adult	Male	38 years	10.839	12.13	-15.30	14.13	40.22	2.85	3.32
To-028 Ind 2	Adult	Male	38 years	8.965	14.59	-14.80	14.92	41.35	2.77	3.23
To-029	Adult	Female	30 - 34 years	0.27	13.30	-14.93	12.47	35.73	2.87	3.34
To-029A	Adult	Female	38 years	1.954	14.02	-13.78	12.64	35.40	2.80	3.27
To-030 Ind 1	Adult	Male	30 - 40 years	4.42	11.47	-16.95	15.1	41.22	2.73	3.18
To-030 Ind 3	Adult	Male	45 - 61 years	0.67	13.61	-13.25	12.53	34.28	2.74	3.19
To-031 Ind A	Adult	Unknown	Unknown	6.24	9.83	-17.07	14.67	41.14	2.80	3.27
To-031 Ind B	Juvenile	N/A	2 years	2.66	8.70	-18.11	4.74	14.17	2.99	3.49
To-031 Ind C	Juvenile	N/A	11-17 years	2.68	9.66	-17.11	11.12	31.29	2.81	3.28
To-034A Ind 1	Adult	Male	50 years	9.301	11.48	-15.82	10.80	31.41	2.91	3.39
To-034A Ind 2	Adult	Female	40 - 50 years	9.63	11.10	-17.51	15.27	42.89	2.81	3.28
To-034A Ind 3	Adult	Female	25 - 30 years	10.351	10.83	-17.08	15.24	42.20	2.77	3.23
To-035 Ind 1	Adult	Male	35 - 50 years	9.957	11.77	-16.63	15.40	43.18	2.80	3.27
To-035 Ind 2	Adult	Male	35 - 40 years	10.124	12.99	-15.20	14.85	41.37	2.79	3.25
To-036 Ind 1	Adult	Male	35 years	2.6	13.21	-14.92	13.03	35.61	2.73	3.19
To-038 Ind 1	Juvenile	N/A	3 years	0.91	13.24	-15.51	12.61	34.76	2.76	3.22
To-038 Ind 2	Adult	Male	35 years	5.42	11.66	-15.60	14.61	39.59	2.71	3.16
To-040	Adult	Male	35 Years	6.38	11.64	-12.64	13.58	39.17	2.88	3.36
To-040	Adult	Male	25-35 years	6.38	11.57	-12.68	13.81	39.86	2.89	3.37
To-040 Ind 3	Juvenile	N/A	9-11 years	10.02	10.86	-16.45	15.20	41.48	2.73	3.18
To-041	Adult	Female	Unknown	8.1	11.37	-17.51	15.64	42.85	2.74	3.20
To-042 Ind 1A	Adult	Male	25 years	7.27	10.88	-15.28	13.94	41.51	2.98	3.47
To-042 Ind 1B	Adult	Male	25 years	10.99	11.23	-15.98	13.34	39.21	2.94	3.43
To-042 Ind 2A	Adult	Female	old adult	9.86	11.28	-15.35	14.95	41.89	2.80	3.27

Original Sample ID	Adult/ Juvenile	Sex	Age	% Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	Atomic ratio C:N
To-042 Ind 2B	Adult	Female	old adult	11.6	11.61	-17.58	15.75	43.60	2.77	3.23
To-043	Adult	Male	30 years	9.64	11.69	-18.05	15.72	42.75	2.72	3.17
To-043 Ind 1	Adult	Male	30 years	13.17	11.99	-15.71	15.11	42.62	2.82	3.29
To-043 Ind 2	Adult	Male	middle adult	10.36	12.12	-16.66	14.24	42.15	2.96	3.45
To-049 Ind 1	Adult	Male	30 years	0.93	15.25	-14.75	4.89	21.95	4.48	5.23
To-049 Ind 2A	Juvenile	N/A	1 year	0.71	21.57	-14.93	0.62	4.40	7.06	8.24
To-049 Ind 2B	Juvenile	N/A	1	0.39	21.72	-14.73	0.98	6.17	6.33	7.38
To-049 Ind 3A	Juvenile	N/A	40 weeks	2.7	18.90	-15.76	1.05	7.25	6.93	8.08
To-049 Ind 3C	Juvenile	N/A	40 weeks	0.35	N/A	N/A	N/A	N/A	N/A	N/A
To-049 Ind 3D	Juvenile	N/A	40 weeks	0.12	12.61	-20.44	2.26	19.63	8.68	10.12
To-049 Ind 3G	Juvenile	N/A	40 weeks	0.82	17.15	-17.50	0.44	3.36	7.67	8.95
To-049 Ind 3H	Juvenile	N/A	40 weeks	0.32	17.91	-15.80	1.25	10.13	8.08	9.42
To-054 Ind 1	Juvenile	N/A	12 years	5.93	11.82	-16.38	13.99	39.03	2.96	3.45
Te-001 Ind A	Juvenile	N/A	40 weeks	0.87	14.79	-17.87	11.17	30.94	2.77	3.23
Te-001 Ind B	Juvenile	N/A	40 weeks	3.3	14.39	-18.13	8.39	22.16	2.64	3.08
Te-002 Ind 1A	Juvenile	N/A	1 month	0.35	11.58	-20.71	1.68	4.36	2.60	3.03
Te-002 Ind 1B	Juvenile	N/A	1 month	0.71	13.47	-20.26	1.10	4.10	3.72	4.34
Te-002 Ind 2	Juvenile	N/A	40 weeks	0.53	12.91	-16.91	2.26	4.33	1.92	2.24
Te-002 Ind 3	Juvenile	N/A	1 month	2.97	11.70	-16.19	13.41	35.97	2.68	3.13
Te-003	Juvenile	N/A	40 weeks	0.56	13.33	-15.29	2.21	6.08	2.75	3.21
Te-003 Ind 2A	Juvenile	N/A	3 months	0.1	12.50	-20.74	1.87	8.17	4.36	5.08
Te-003 Ind 2B	Juvenile	N/A	3 months	1.09	10.94	-17.70	0.87	3.14	3.59	4.19
Te-004 Ind 1A	Juvenile	N/A	1 month	2.82	14.91	-16.82	10.81	30.23	2.80	3.26
Te-004 Ind 1B	Juvenile	N/A	1 month	0.26	14.62	-17.30	8.45	22.78	2.70	3.15
Te-004 Ind 2A	Juvenile	N/A	3 months	6.67	12.53	-16.24	9.71	32.48	3.34	3.90
Te-004 Ind 2B	Juvenile	N/A	3 months	1.15	12.47	-16.74	5.52	15.01	2.72	3.17

Original Sample ID	Adult/ Juvenile	Sex	Age	% Collagen	d15N	d13C	wt %N	wt %C	wt ratio C:N	Atomic ratio C:N
Te-004 Ind 3	Juvenile	N/A	9-12 years	4.95	11.13	-17.61	13.59	37.38	2.75	3.21
Te-005	Juvenile	N/A	13-15 years	2.18	8.90	-17.49	12.87	35.44	2.75	3.21
Te-007/008 Ind 1A	Juvenile	N/A	2-3 years	6.35	13.75	-15.01	15.80	43.76	2.77	3.23
Te-007/008 Ind 1B	Juvenile	N/A	2-3 years	2.65	13.23	-15.23	6.00	17.25	2.87	3.35
Te-011 Ind A	Juvenile	N/A	40 weeks	3.1	11.26	-18.22	12.91	35.87	2.78	3.24
Te-011 Ind B	Juvenile	N/A	40 weeks	0.02	12.13	-18.20	7.15	23.38	3.27	3.81
Te-013 (a)	Juvenile	N/A	40 weeks	1.11	13.28	-16.53	11.98	33.24	2.77	3.24
Te-013(b)	Juvenile	N/A	40 weeks	1.04	14.24	-14.60	2.48	6.92	2.79	3.26
Te-014a	Juvenile	N/A	40 weeks	1.1	14.25	-16.47	4.60	12.64	2.75	3.21
Te-014 Ind B	Juvenile	N/A	40 weeks	1.05	14.38	-16.59	1.71	5.11	3.00	3.49
Te-015 (2a)	Juvenile	N/A	1 month	4.64	12.09	-15.07	10.91	29.98	2.75	3.20
Te-015 (2b)	Juvenile	N/A	1 month	4.34	12.00	-14.96	11.45	31.67	2.76	3.23
Te-017a	Juvenile	N/A	1 month	6.52	13.76	-14.88	14.52	41.54	2.86	3.34
Te-017b	Juvenile	N/A	1 month	9.55	13.38	-15.91	13.18	36.46	2.77	3.23
Te-019	Juvenile	N/A	7 years	7.69	12.05	-16.83	15.42	42.54	2.76	3.22
Te-026	Juvenile	N/A	40 weeks	0.28	11.30	-15.92	15.07	45.17	3.00	3.50
Te-033	Juvenile	N/A	8 years	2.26	10.62	-17.22	14.34	39.86	2.78	3.24
Te-034	Juvenile	N/A	16 years	3.13	11.50	-16.32	14.49	39.9	2.75	3.21
Te-035	Juvenile	N/A	1 year	0.45	10.94	-18.29	5.87	20.46	3.49	4.07
Te-057	Juvenile	N/A	1-3 months	0.44	11.34	-15.42	15.16	42.26	2.79	3.25
Te-063	Juvenile	N/A	4 years	2.12	10.29	-18.67	11.48	32.47	2.83	3.30
Te-066 IND A	Juvenile	N/A	38 weeks	6.23	14.05	-14.57	8.54	22.57	2.64	3.08
Te-066 Ind B	Juvenile	N/A	40 weeks	2.335	14.01	-14.34	14.08	38.75	2.75	3.21
Te-070	Juvenile	N/A	2 years	8.23	11.24	-16.90	14.00	40.68	2.91	3.39

* Yellow indicates samples that were not preserved

**Green indicates a duplicate sample

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